Chapter Five

Interpretation of the study on 'Induction of Stem Cells from Somatic Cells'

[Editorial Comments]

The study on 'Induction of Stem Cells from Somatic Cells' is the technical foundation of 'Human Body Regenerative Restoration Science'. It is also the first discovery in revealing the mystery of regeneration. Its research process reflects our scientific beliefs and methods, as well as our new philosophical concept of symbiosis between holistic and individual organisms. Without this new philosophy, it will be impossible to reveal the mystery of body regeneration and to establish the new system in life science. My achievements on the induction of stem cells from somatic cells and skin regeneration in situ were announced and published in 2000. For scientists' and readers' benefits, and to push the development of this new science forward, I, together with the journalist from *Science and Technology Daily*, have decided to interpret this scientific discovery and its process through this report in order to truthfully reveal the mystery of the study on 'Induction of Stem Cells from Somatic Cells'. In this report, by the philosophy of harmony between holism and individualism, we predicted and analyzed the prospects of the front line research in life science. During the last nine years, our scientific research in transformation of somatic cells into stem cells was clinically expressed in a wide range and formed a new system in body regeneration science. Our prediction on life science front line research, such as stem cell research, was proved to be true. Although the following report was published nine years ago, it still represents the most advanced study philosophy and methods in the area of life science. In order to help people remember and understand this discovery, we included this full length documentary report, which was written by Xinhua Yan, a journalist from *Science and Technology Daily*, and published in November, 2000, into this book. The full text is as follows.

Challenge the Frontier of Life Science

- Interpretation of Rongxiang Xu and his stem cell research

Xinhua Yan, journalist of *Science and Technology Daily*

[Editor's note] In the current research of life science, the enthusiasm in gene has shifted to stem cells. In general, western scientists believe that stem cell research will provide broad prospects for clinical medicine. Stem cells, especially in terms of tissue engineering, are believed to be able to resolve the long-time disturbing clinical difficulties, such as insufficient tissue and organ supplies, and immune rejection. Consequently, the free replacement of diseased tissues and organs by artificially cultured counterparts will be achieved.

Guided by western scientists, the international research was initiated and focused on *in vitro* stem cell culture. At the same time, based on the achievements in the fully

functional repair of burn tissues, Chinese scientist, Rongxiang Xu, uniquely established *in situ* stem cell culture mode. The establishment of this approach separated the international stem cell and tissue engineering research into *in situ* and *in vitro* groups; or eastern and western groups. Since Rongxiang Xu's stem cell research was derived from his successful clinical practice, it provided realistic guidance and reference for international stem cell research.

Today, in order to describe Rongxiang Xu's stem cell research, and to interpret the significance of his research for international stem cell community, our paper specially published this full length report written by our journalist.

[Introduction]

Back in 1991, American burn specialist Barbara repeated the experiments in the United States to validate the therapeutic effects of MEBT (Moist Exposed Burn Therapy) techniques invented by Professor Rongxiang Xu. As obtained by his Chinese peers from their practice, Barbara's results again showed that even for patients with severe burn, not only were their lives in no danger, but the skin wound healings were also perfect without scars.

As the first traditional burn specialist who had known Rongxiang Xu in early years, and had supported and advertised Xu's MEBT techniques, Barbara suggested that they worked together to identify the mechanisms behind the amazing therapeutic effects of MEBT techniques. Barbara predicted: □Once the puzzle is solved, this achievement will definitely win the Nobel Prize.□

In 1998, Gordon, another American researcher from the Office of Alternative Medicine made the same prediction. He was invited to attend *International Conference of Integrative Medicine* in China. During the conference, he directed Rongxiang Xu's speech and highly appraised the MEBT techniques founded by Rongxiang Xu as a miracle in medicine.

During the dinner following the conference, Gordon expressed the intention to work with Rongxiang Xu to solve the puzzle behind the techniques. He said: □I have no doubt on the effects of this MEBT technique. The key point is that you have to clarify the mechanisms of skin healing without scar, which will be a huge contribution to science. In the field of molecular biology research, the Unite States has the most abundant theories, technologies, funding, and experimental resources. Based on my experiences and influence in the medical community, I believe that this research will be strongly supported in the United States. If the mechanisms of physiological healing in burn wound can be explained, it will have great chance to win the Nobel Prize!□

Seven years apart, the two American researchers made incredibly similar comments. They both used the same specification Nobel Prize, to describe the significance of

clarifying the mechanisms of physiological healing in burn wound under MEBT techniques.

If the mechanisms of physiological healing in burn wound by MEBT were indeed clarified, could it win Nobel Prize? Individual opinion here weighed not much. Although Barbara and Gordon both said so, we could not be too serious about it. Firstly, both American researchers were not the members of Nobel committee and not directly involved in the selection. Secondly, even if they were the members, there were still unpredictable factors in the selection process. There were previous instances in which Nobel Prize had been awarded to those who should not be awarded, and had not been given to those who should be given. Nobel Prize had indeed left some regrets to the world. The famous mathematician Chengtong Qiu recently mentioned that Nobel Prize is not the only measurement to evaluate scientific achievement.

We should not be too serious about winning or not winning Nobel Prize, but we should be serious about how to evaluate the significance of revealing mechanisms of physiological healing in burn wound. This was a realistic question, since Rongxiang Xu had announced to the world that he had obtained the results on mechanisms of physiological healing in burn wound.

In the afternoon of August 8th, 2000, the drizzling rain brought autumn coolness to the hot city of Beijing. Hundreds of researchers and reporters were gathered in Beijing Hotel to witness this historical moment, when Rongxiang Xu announced that his research team had completed the research on *in situ* physiological repair of burn tissues by skin stem cells.

How to estimate the significance of this research finding? By their professional instincts, the press smelled the major scientific news behind it. However, tissue engineering by stem cells was in its infancy at that time, the insufficient background information and knowledge made it difficult to accurately evaluate this finding. As a result, *Beijing Evening News* mistakenly stated that Rongxiang Xu's results were listed on Journal of *Science*'s Top Ten List of the Best Scientific Advances when actually another stem cell research was chosen.

Nonetheless, the mistake made by *Beijing Evening News* reminded people to think over: the American scientists only completed isolation and proliferation of stem cells *in vitro* without clinically applying it for disease management. A mere finding like this could be honored as the first on the top ten scientific advances, then how the accomplishment, such as by using stem cells, Chinese scientists successfully regenerated skin *in situ* on burn wound tissues, and healed severe burn wound without scar, could be honored!

It was not our main focus whether there was definite correlation between Rongxiang Xu's achievements and Nobel Prize or Top Ten Scientific Advances by *Science*. What we would really want to discuss here was the significance of Rongxiang Xu's latest

achievements in burn medicine, life science, and the future of human being.

Together, let us interpret Rongxiang Xu and his stem cell research.

[Could the research on 'tissue engineering from *in vitro* stem cell culture' fulfill the dream?]

In November, 1998, Thomson, University of Wisconsin, and Gearhart, Johns Hopkins University School of Medicine, published their researches in the Journal of *Science* and *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, respectively, to announce their research findings on human embryonic stem cells. Thomson's work was to isolate stem cells from inner cell mass of blastocyst formed during early human embryonic period, and to culture embryonic stem cells *in vitro* for continuous proliferation, and finally to produce a stem cell line; Gearhart's work was similar, with the difference that the stem cells were isolated from the tissues which will eventually develop into testis and ovary.

The above researches, along with other advances in stem cell research, were listed by the worldwide famous journal *Science*, as the first of Top Ten Scientific Advances, the second on the list was human gene research.

Closely after the two American researches, stem cell fervor emerged worldwide, with the scientific world, the government, and the commercial society all involved.

At the government level, some of them have loosed up the previous ethic bans in life science research. American president Clinton declared on 23rd August that the U.S. government had decided to support the research in human embryos. He stated: America will not give up human embryonic research which has great potential in saving lives, improving life quality, and treating disease. The British government at the same time, although cautious and conservative about life science all the time, formed a medical investigation team to look over the supportive opinions. On 16th August, the British government endorsed Donaldson, the chief medical officer's report on using embryonic cell cloning to perform medical research, and also proposed to loosen up the regulation on cloning in order for scientists to isolate cells from embryos, produce skin and other human tissues and organs, and finally to cure the diseases cureless at that time. The report would be submitted to senate for voting later that year.

The commercial organizations were also attracted. In a short period of time, numbers of companies focusing on stem cells were established, including eight companies in the United States, at least one in Britain, Australia, and Japan with certain influence. Among them, Geron Bio-Med Ltd. was the most famous company which had the patents from Thomson's and Gearhart's research, and acquired Roslin Bio-Med of Roslin Institute well-known for its clone of 'Dolly the Sheep'.

Scientific institutions were all ready as well. There were files indicated that nearly all

American universities had established stem cell research unit. Reports from U.K. showed that three bio-engineering teams from the United States and Canada were making an ambitious global plan: to artificially produce heart, liver, and kidney in the laboratory with the expenses of three billion British pounds.

The fervor of stem cells might be caused by two factors, one was the nature of stem cell itself, and the other involved life ethics.

As the nature of stem cells, they have great potential in clinical application. Stem cells are non-fully differentiated, immature cells with the potential to continually differentiate. Under the appropriate conditions, stem cells can become pluripotent cells which will be able to produce some of the tissues and organs, or even become totipotent cells which can produce a complete organism. At the beginning, cells with this primitive differentiation capacity are thought to be embryonic stem cells that can be isolated only form early embryos. The current knowledge about human stem cells shows that zygote divides continuously in maternal uterus for about ten days before developing into a cystic structure called blastocyst. Within blastocyst there is an inner cell mass containing about 100 embryonic stem cells the basic cells developing into adult tissues at the later developmental stages. Every cell, through proper regulation, has the ability to generate all 210 cell types of human adult tissues.

American scientists had successfully isolated and cultured stem cells to indefinitely proliferate *in vitro*, which was a big step towards the clinical application. Currently, some serious disorders, such as heart disease, diabetes, renal disease, and Parkinson's disease were constantly disturbing the clinical practice. Satisfying treatment was yet to be found. Therefore, some scientists recommended the □therapeutic cloning□ approach: transfer the nuclear of patient's cell into denuclearized maternal cell, reactivate the reprogrammed cell and produce stem cell lines, and induce these cells to become the cell types needed by the patients. Because these cells are coded by patients' own nuclear DNA, their phenotypes supposed to be unchanged, therefore will presumably not be rejected by patients' immune system. In addition, the indefinite proliferation of pluripotent stem cells will provide adequate supplies for tissue repair.

Despite that, this achievement in stem cell research certainly provided a new approach for clinical treatment of certain diseases. It triggered imagination on tissue engineering instead of on cellular therapy in which the hope was placed on stem cells, i.e. by regulating stem cells to regenerate tissues and organs *in vitro*, and then diseased tissues and organs could be repaired by transplantation.

Currently there are three approaches for tissue repair: autologous tissue grafting, allogenous tissue grafting, and artificial replacement. All of these approaches had difficulties with immune rejection and insufficient donor resources. Scientists believed that stem cell approach was the most advantageous way to avoid the above limitation. They outlined an exciting picture: stem cells are cultured and directionally differentiate to produce viable tissues and organs. In the future, doctors would treat diseases like mechanics repair machines, just to change the parts that are not working.

According to files from American Patients Association, there were nearly 100 million patients waiting for treatment. To the perceptive commercial society, this was tens of billions of US dollars worth of business opportunity behind stem cell tissue engineering.

From life ethic point of view, the potential clinical application of stem cells would not be strictly restrained by ethic regulations. Comparing to human cloning techniques, stem cell research was associated with far less serious life and ethic setbacks. Although embryonic stem cells came from embryos taken as live organism by western people, embryos were the most primitive organisms on which the manipulation was under less ethic pressure than on adults. It was relatively acceptable to produce human tissues by stem cells from embryos. On the contrary, it was intolerable to use cloning human as the supply of clinical practice.

Bearing the enormous hope of human being, accompanied by cheers, stem cell research started its journey like a ship heading into the eternal sea.

Excessive expectancy might cover the unavoidable complexity of stem cell research behind the beautiful dream. It seemed that as long as the proposed research continues, it would reach the brilliant far shore no matter what.

Could stem cells really fulfill the dream of *in vitro* regeneration of human tissues and organs as imagined and expected?

The most extraordinary hope usually comes with the most vigorous challenge. Like the development of any other science, in order to fulfill the dream of humankind ,study of stem cell tissue engineering *in vitro* must face the reality and meet the challenge unconquerable at times,.

Before facing the large amount of potential challenges, western stem cell tissue engineering research must face a real challenge, which was not simple. This challenge, coming from an ancient country in the east, was Rongxiang Xu and his stem cell research.

[The dream of the stem cell has been fulfilled in an eastern country]

1. To explain MEBT (Moist Exposed Burn Therapy) techniques by empirical theory itself was not enough and satisfactory.

In the early 90s of 20th century, Rongxiang Xu completed his MEBT techniques, which shocked the clinical burn society. His therapeutic techniques had entirely changed the current state of burn treatment. By applying MEBO, his uniquely invented medication, and moist techniques (MEBT), wound healing without scar formation could be achieved even in severe burn cases.

These revolutionary therapeutic effects strongly shocked the traditional burn treatment techniques based on burn surgery. Rongxiang Xu and his medical techniques became the center of debate in traditional burn medicine in China. Some researchers denied the MEBT techniques, blamed Rongxiang Xu as a liar, and his techniques as a scam. On the other hand, other researchers believed that the MEBT techniques were the most advanced achievements in burn medicine. They became advocates and applied MEBT techniques themselves.

Why, among the same group of traditional burn medicine researchers, was there a totally contradicted opinion towards the same technique?

The key question based on which the opponents opposed, or the advocates supported the techniques, was about the mechanisms behind the techniques.

The critics against the moist techniques were based on experiences and traditional theories. In accordance with experiences, with the exception of epidermis that could automatically undergo physiological healing without scar formation after injury, injured deeper skin tissues were not able to heal without scar by themselves. The rationale provided by traditional theory was that the basal stem cells residing in epidermis contributed to epidermal healing after epidermal injury. When the epidermis was injured, basal stem cells were automatically initiated to differentiate into tissues to repair. Even without injury, the old tissues needed to be replaced by new tissues during normal epidermal metabolism, which was carried out by basal stem cells also. Basal stem cells were mostly located in epidermis, with a few found in dermis. As a result, epidermal repair became impossible once the burn was deep into dermis, fat tissues, or underlying muscles where there were no stem cells. Based on this established theory, some researchers confidently concluded: Whatever Rongxiang Xu claimed by his wish that he can cure, it was not real. Nobody can make achievement against the principles of life.

The fact that during the nearly ten years of development, MEBT had successfully treated millions of patients was still not enough to change the opponents' attitude. Their stubbornness should not be blamed, since the key lay in the fundamental theory. In the time of molecular medicine, the principles of life could be thoroughly and accurately elucidated on molecular level, whereas the approach of moist therapy tried to explain the mechanisms by empirical theory, which was clearly equivocal.

Nevertheless, the supporting researchers, who believed that the fact is the first priority and the theory is only the second, felt the techniques were unfairly treated. The contribution of Rongxiang Xu's MEBT was not merely raised burn medicine to a level never seen before; more important, it revealed through clinical practice that deep injured skin is able to undergo physiological healing. This was a revolution not only in therapeutic medicine, but also in human recognition. Without moist therapy techniques, our understanding on skin tissue generation would not be intrinsically profound or superficially comprehensive.

MEBT could not be clearly explained in theory, which was not the fault of the techniques. Quite the contrary, it was due to the insufficient understanding by traditional theories in new phenomena, which did not meet the needs in reality. The reality required the tradition to confront the reality, the challenge, and the innovation. Some researchers already sensed the imperative need to commence study on the mechanisms of MEBT techniques and recognized their significance for the future, which will not be limited in burn medicine. The mechanisms would be a 'nuclear fission' involving the entire medical field and the future life science. This was likely the reason that the two American researchers, Barbara and Gorden, used 'Nobel Prize' as the specification to assess the value of this study.

Rongxiang Xu, the inventor of MEBT techniques, had been diligently strived after the mechanisms behind MEBT for years. After the birth of MEBT, Rongxiang Xu had further improved his techniques, and simultaneously initiated the study on the mechanisms behind skin wound healing without scar. As early as in 1989, on the first issue of the journal *The Chinese Journal of Burns Wounds & Surface Ulcers* founded by himself, Rongxiang Xu and his research team published their research paper, in which the special cell growth observed under optical microscope was reported. In the last quarter of 1991 the full scale research was ready to launch.

2. Expedition of stem cell army was aborted halfway through

On October 25th, 1991, Barbara, the famous burn specialist from Hackensack University Medical Center informed Rongxiang Xu through international phone conversation about the repeated experiments he performed on BC4D, the core medication of MEBT. The following were the summary of this conversation:

□You visited America in December, 1990. Before leaving, you gave me the study about MEBO. After 1 year efforts, we finally got the results now. By using MEBO you left, we performed following experiments, and the results are satisfying.

The first experiment was to use MEBO to study cell culture *in vitro*. In short, the experimental methods were as follows: using two groups of epithelial cells in the test tubes all filled with media. MEBO was added into only one group of test tubes (test group). The cell culture results were then compared. The results were remarkable. In the test group, the epithelial cells transformed into mature basal cells with rapid growth, which was determined by precision apparatus. These results, could not be obtained by using any other drug, were a significant world-class breakthrough!

The results of the second experiment: putting dermal explants into cell culture media with MEBO, which resulted in rapid regeneration of residual hair follicle gland epithelium and hair follicle gland under MEBO effects, the morphology of regenerated hair follicle gland and hair follicle appendage were unchanged. All of these were observed directly through microscope. Previously, people had only prefigured the similar change by the same pattern of hair follicle regeneration. However, the growth of hair follicle epithelium was extremely slow so that nobody

was able to describe, nor to observe the regeneration directly. But, after MEBO application, considerable increase of the growth rates could be observed directly. This was also a big international breakthrough.

The results of the third experiment regarding molecular biology: we tried to study the 'regenerative' gene engineering of regenerated tissue cells applied with MEBO, and measured the DNA and RNA with experimental tools. The experimental results showed: after the application of MEBO, the number of DNA and RNA in the tissue cells were significantly increased, which indicated rapid cellular division. The results, as important as those above, were never attempted and obtained before by anybody in the world.

These results had major impacts on the FDA approval of MEBO entering into American market. Since the experiments were performed in FDA approved laboratory and even funded by FDA, they were totally reliable.

Even though Rongxiang Xu had completed the experiments performed by Barbara, the information coming across the sea still brought the urgency to him. After the phone conversation, Rongxiang Xu reorganized related files and went to the science department of China's Ministry of Health. He reported to the persons in charge and suggested to initiate full scale study under government supervision on the mechanisms of moist therapy techniques. His suggestion drew immediate attention from both the department and the ministry.

On October 30th, 1991, directed by the science department of Ministry of Health, a large conference involving researchers in basic medical science from various research authorities, including Chinese Academy of Medical Sciences, Chinese Academy of Preventive Medicine, and Beijing Medical University, was held in Capital Hotel. During the conference, Rongxiang Xu introduced the extraordinary effects of MEBT on physiological regeneration in burn skin tissues. He also recommended the subjects need to be explored further, which included: 1) epidermal regeneration by sweat gland epithelium, 2) full regeneration of dermis and appendages, and 3) the study on the pattern of rapid DNA change.

The report by Rongxiang Xu genuinely encouraged these researchers in basic medical science. Within one month, a detailed study design was prepared and submitted by nearly 50 prominent researchers in basic medical science of China, with up to 150 researchers involved. Facing the thick folders of study description, Rongxiang Xu seemed going back into the ancient world, genuinely felt the greatness of the war of the century like an epic hero.

What a great era, what an encouraging plan, what an exciting study!

While the group study on the pattern of skin cell growth was underway, the battle began and the curtain was rising. Suddenly, Rongxiang Xu was heavily attacked by traditional burn medicine in China.

Though Rongxiang Xu eluded from this adversity by the support from Ministry of Health and the State Council of China, his expeditionary army was dismembered. During the following days, Rongxiang Xu experienced a series of complex and risky events consisted of critics and supports, investigating and being investigated, and frame and counter-frame. He was forced to be involved in a struggle in order to back his MEBT techniques up. The research plan of skin tissue cytology had to be buried deeply in his heart.

Dying before conquer, mourning ever since.

At the end of 1994, Rongxiang Xu's experience received attention from the newly appointed Health Minister Wenkang Zhang, who inquired to Rongxiang Xu about the research status of the mechanisms in moist therapy techniques. After acknowledging that Rongxiang Xu's study was terminated due to the academic dispute, Wenkang Zhang resolutely instructed that the study could not be stopped; the Ministry of Health would create a healthy environment to support Rongxiang Xu's research.

After a cold winter, Rongxiang Xu was welcomed by the warmness of spring. On May 22nd, 1995, he announced the initiation of skin stem cell research on Chinese CCTV Xinwenlianbo (CCTV network news).

3. To locked up 'induced human keratin 19 positive cells'

Without a large-scale corps, without massive ceremony, Rongxiang Xu, along with his research team with less than 10 people, disappeared from press and public view. On April 1st, 1996, he and his research team left Beijing and headed to Xiangfan, Hubei Province where there happened to be large numbers of severe burn patients receiving the treatment of MEBT techniques. From there, his basic and clinical cytology combined study on skin regenerative mechanisms began.

This time his opinion about the study, comparing with five years ago, had changed dramatically. Despite the time wasted in the struggle for the survival of his study in the last five years, the experience also gave Rongxiang Xu a chance to be composed. From fervor to calmness, the impatience was removed by the time, and the hidden correlation of nature gradually emerged in his mind.

During the five years, Rongxiang Xu had never stopped thinking about the skin regenerative mechanisms. Firstly, these thoughts surfaced as inspirational bursts, full of intellectual sparkles, splattering everywhere, rich and disorderly, just like the wide variety of more than 50 study subjects submitted by other scientists. Gradually, his mind focused on only one subject---stem cell.

Rongxiang Xu's mind was controlled by a cytological concept of keratin 19 stem cells. Initially, he promptly noticed this cell not through logical deduction, but an

inspiration. Nonetheless, he kept himself focusing on this cell and was afraid that it might disappear from his fissile mind. Later, through rational analysis, he predicted even before confirmed by experiments: the basic causative substances for the complete physiological repair of deep injured skin were most likely the keratin 19 positive stem cells. Once the conclusion was drawn, his mind was locked in an unshakable state.

Back in 1989, Rongxiang Xu directly observed through optical microscope that after MEBO application, the cells in the burn tissues underwent specific changes. This was consistent with the *in vitro* results obtained by Barbara. Once MEBO was applied, large amount of cells proliferated in the burn wound and congregated to form a cell mass. This change could not be seen in the wound without MEBO application. When the tissue slide was sent to *Chinese General Hospital of the People's Liberation Army*, the authoritative cytologist exclaimed: 'what is this tissue, how can embryonic tissue cells come to the burn skin wound?!'

Based on the knowledge of skin tissue cells provided by known basic research, Rongxiang Xu acknowledged that the detected skin epidermal cells in various differentiation states contain 5 types of keratin: human keratin 1, 10, 9, 16, and 19. Different cell types have different functional expression. Cells containing positive keratin 1 and keratin 10 are mature epidermal cells with the ability to produce harder stromal cells, their proliferation and differentiation ability are almost exhausted; Keratin 9 and 16 positive cells are immature intermediate cells appeared in the migration process of epidermal stem cells with the ability to differentiate into keratin 1 and 10 positive cells; keratin 19 positive cells are epidermal regeneration cells with the most potential differentiation ability.

All of the above cells contribute to the growth and repair of human skin at different stages. However, except keratin 19 positive cells, all other cells, including keratin 1, 10, 9, and 16 positive cells are intermediate cells naturally residing in epidermis only. Therefore, none of these cells, but keratin 19 positive cells can complete skin regeneration in deep burn wound.

Still, there are mental barriers that make the unquestionable acceptance of keratin 19 more difficult than it should be. Based on what we already know, keratin 19 positive cells are embryonic cells that can only be found in the process of embryo development from zygote in the maternal uterus. By the development of embryo, keratin 19 positive cells will differentiate into other cell types. To identify keratin 19 positive cells, firstly we must find the possible residence, i.e. try to find them in epidermis, dermis, fat layer, and even muscles, where are thought to be impossible for them to reside; in addition, we have to track the alteration and transformation of keratin 19 cells. If we can identify the division, proliferation, and differentiation of keratin 19 cells into keratin 1, 10, 9, and 16 positive cells in the skin healing process in deep burn wound after MEBT, then we can be certain that the keratin 19 cells are found.

4. Conquering stem cell was easy as pie

Theory-first approach made the practice straightforward. Previously it was believed that a full-scale, joint research with multiple subjects was essential to achieve the goal. Now it seems not necessary. The subject was simple and clear: to clarify the cells with keratin 19 positive expression. This enabled a simple research unit to finish the study, which was originally thought a huge project and requiring substantial personnel resources. The research unit was further divided into 2 teams. One was to strictly supervise the clinical practice to make sure that in order to perform basic research observation, patients' biopsy samples will be accurately obtained as scheduled. The other team was to choose the best laboratory in which the transformation of stem cells would be followed by the most advanced cell tracking technology.

Sometimes destiny counts. Coincidentally, there was a group of severe burn patients in Xiangfan, Hubei Province, which provided a valuable chance for basic stem cell research. Rongxiang Xu himself participated in the rescue and treatment of each patient. Furthermore, he guided researchers to make pathological slides in accordance with the planned specification. At the same time, he had to travel back to Beijing once in a while to discuss experiment designs and methods with researchers from *China Medical University*. In one occasion, right after arrived at Beijing airport and turn his mobile phone on, he received a phone call from the clinical center in Xiangfan, which informed him that there was a patient in critical situation and the hospital physician was unable to handle. Rongxiang Xu immediately took another flight heading back without hesitancy. Just when the hospital physician decided to give up on that patient, Rongxiang Xu arrived. By his management, the life threatening symptoms in this patient was relieved soon.

There were also setbacks during the experiments in Beijing. Initially, researchers found that the immunofluorescent results showing no keratin 19 positive cells expressed on the slides. Rongxiang Xu was surprised by the results sent to his office. Since he had unbreakable confidence on keratin 19, he did not believe the results. There must be something wrong somewhere. It was hard to find any error through either purely theoretical deduction, or the examination of the experimental procedures applied during the observation. Rongxiang Xu started to analyze the experimental results and found that they were far from normal. The cells on the slides showed mutation generated by passage and were not the original. This indicated that there were errors in the sampling process.

Going back through the sampling methods, Rongxiang Xu found that what he proposed was possibly true. He discussed this with Zenglu Xu, professor of *Chinese Academy of Medical Sciences* affiliated *Peking Union Medical University* and a member of study subject validation team, and decided to immediately freeze the slides after sampling and reserve the slides in liquid nitrogen. Once the storage condition was changed, the experimental observation became uncomplicated.

Next step was to perform indirect staining with biotin avidin DCS system on the biopsy slides. Indirect staining with biotin avidin DCS system was the most advanced cell staining technique in the world. It could show specific mark through fluorescencent display and therefore, was the ideal cell tracking method.

Firstly, in order to determine the existence of keratin 19 positive cells in the treated burn tissues, researchers selected a monoclonal antibody, mouse antihuman keratin 19 antibody as the tool. During the experiment, the normal skin taken as control and was compared to the burn skin treated by MEBT, to see if there was any specific response. Researchers found no keratin 19 positive cells in the normal skin, which met the anticipation. On the other hand, keratin 19 fluorescent positive cells were found in MEBO treated burn tissues 24 hours after application.

In addition, observation on treated burn tissue cells continued. Four days after treatment, researchers found increased number of keratin 19 cells in sweat gland, capillaries, and the tissues surrounding hair follicle. Seven to fourteen days after treatment, the number of keratin 19 cells reached the peak. Twenty-one to twenty-eight days after treatment, the cell numbers began to drop. These results were consistent with clinical and histological results obtained from treated burn skin.

The experiment achieved the initially established objective to identify keratin 19 positive stem cells, and to track their proliferation and differentiation. The mechanisms of burn skin tissue regeneration and repair emerged.

I. The experiment demonstrated that human keratin 19 positive cells, the most primitive embryonic cells, could be produced in the residual burn tissues; the change of the numbers of keratin 19 positive cells indicated that these cells had the ability to continuously differentiate and proliferate;

II. The results from immunofluorescent stain showed that keratin 19 positive cells could, after migration and division, generated other types of keratin positive (keratin 1 and 10) cells contained in mature epidermis, which sufficiently proved that burn skin tissue regeneration and repair were carried out by these human keratin 19 positive cells;

III. Researchers also primarily confirmed the initiation and regulation model of human keratin 19 positive cells. Research showed that cells were released from stationary phase. The firstly activated protein was cyclin D, which was expressed only by the stimulation of growth factors. The cells in eukaryotic organisms were regulated by cycle protein cyclin D1/CDK4 complex at G1 phase to determine whether to enter proliferative state or withdraw from the cycle. Thus, the initiative and regulatory effects of MEBT techniques on stem cell division and self renewal, and regeneration was proved.

IV. Researchers also discovered that fibroblasts, vascular tissue cells, and skin neural cells were able to continuously proliferate and generate skin by either themselves, or by the interaction between them. The detailed results would be gradually published in the future.

In April, 1998, the research team led by Rongxiang Xu primarily completed the study

on identifying the mechanisms in physiological healing of injured skin after treated with MEBT/MEBO. The beautiful dream of treating clinical diseases with stem cells was fulfilled in the ancient east.

[Challenge the epic magnificent dream with iron cold reality]

1. In situ culture opened a new approach of stem cell research

The results of Rongxiang Xu's stem cell research had historic significance for life science research and development worldwide. At academic level, his research heralded the new approach of *in situ* stem cell culture, and therefore changed the overwhelmingly biased structure of worldwide stem cell research. At therapeutic level, through *in situ* culture of stem cells, the research fulfilled people's dream of treating disease with stem cells. Human medicine had stepped into stem cell era. Rongxiang Xu's success in clinical practice provided a new approach for the development of human medicine in 21st century.

At academic research level, international stem cell research was awfully imbalanced and dominated by *in vitro* stem cell culture established by western researchers. The emergence of Rongxiang Xu's achievements in stem cells changed the current situation by dividing the stem cell research into western and eastern groups, and *in vitro* and *in situ* approaches. An innovative eastern vs. western pattern appeared.

As said by the western *in vitro* approach, the trilogy of stem cell research included: firstly, isolation of stem cells; secondly, *in vitro* culture with nutritional media and directed indefinite differentiation; and the last, transplantation of cultured cells, tissues, or organs into patients to cure disease.

Alternatively, the approach founded by Rongxiang Xu introduced *in situ* replication by stem cells. By this approach, all processes were completed within the *in situ* physiological environments and as a result, stem cell isolation and directed differentiation, and transplantation were all unnecessary.

In situ contradicting with *in vitro*, it was natural to misunderstand that the *in situ* concept was created against the western concept. This was not the case. In fact, there was no correlation between them. The *in vitro* culture in western approach was based on a hypothesis that had not been confirmed. On the contrary, Rongxiang Xu's in situ stem cell culture was derived from his successful clinical practice.

Rongxiang Xu's *in situ* culture approach was consisted of two aspects: clinical medicine & therapy and stem cell research. Before the basic research in revealing skin stem cells was commenced, the breakthrough on stem cells was more significant, since it was the key to clarify the foundation of moist therapy techniques. However, once the results of stem cell research became available, comparing to MEBT techniques, which became increasingly important, the significance of stem cells was reduced.

Why? The reason was that moist therapy techniques were invented before the stem cell mechanisms were discovered. Furthermore, if moist therapy techniques were 'initiative' research; then the stem cell research was rather 'passive'. The motivation for Rongxiang Xu to study stem cells was not to further clinically treat disease, as anticipated by western researchers. Quite the opposite, he started stem cell research just in order to reveal the mechanisms behind his successful moist therapy. So the clinical treatment first, followed by stem cell research. To be exact, *in situ* stem cell culture objectively summarized and described moist therapy techniques on molecular level. Another reason was that during the process of injured skin healing by moist therapy techniques, although the physiological skin regeneration and repair was achieved by the dynamic functions of stem cells, it was undeniable that those functions were not generated by stem cells themselves. Instead, they were initiated and maintained by moist therapy techniques and their supportive conditions and environments. Thus, moist therapy techniques significantly contributed to *in situ* stem cell culture and function performance.

It could be said that moist therapy techniques were fundamental, stem cell *in situ* culture was derivative in Rongxiang Xu's research. Without moist therapy techniques, there would be no whole new recognition on stem cell activities, and even less appreciation on stem cell *in situ* replication regulated by in situ culture of stem cells. If so, then all the praises and hopes on stem cells would be wonderful dreams only. Only because moist therapy techniques, could people truly experience the vigorous vitality of stem cells, and use stem cells to cure diseases the first time.

To be objective, if to evaluate western and eastern groups by clinical application of stem cells in clinical medicine, then Rongxiang Xu was already ahead of others and reached the destination. In stem cell research, he came from the end back to the starting point where his western peers were standing. The achievements in stem cell research carried out by western researchers only represented the beginning. They were still in the first chapter of the trilogy. When facing the confusing and constantly changing future, even the researchers themselves were not certain about their chance of success. They were far, far away from the destination of repairing and regenerating tissues and organs with full function *in situ*. It was not exaggeration to call their research a dream.

The first clinical breakthrough by Rongxiang Xu's moist therapy techniques provided a firm ground on which his stem cell research was developed further, which offered us the precious first hand knowledge to more comprehensively recognize stem cells and life activities. It also encouraged new way of thinking about stem cells, guided stem cell researchers toward the direction of life science and life study, and assisted researchers by successful experience to explore the new approach in therapeutic medicine of 21st century.

2. In vitro stem cell tissue engineering study was standing at the crossroads between the history and the future

Why the results from the two stem cell researches that were started almost synchronically were so different? One was so lucky with rich intellectual inspirations as God given; the other was crawling in the dark.

On the surface, it seemed that different methods produced different outcomes. *In situ* or *in vitro*, the difference between western and eastern researchers was deeply rooted. This difference was demonstrated by the different observation methods applied by Rongxiang Xu and Barbara when investigating the effects of MEBO on cells and tissues. Rongxiang Xu observed the effects of MEBO on stem cells by sampling *in sit*u tissues. In contrast, Barbara took cells into the test tube and cultured, then observed the MEBO effects.

Methods were human behaviors. They reflected the difference in intrinsic recognition, which was directly stated by understanding on specific objects, such as the recognition of cell nature. Indirectly, it was expressed by different philosophical understanding on abstract objects, such as the concept of life.

In situ and *in vitro* reflected the intrinsic difference of understanding on stem cell activities: Rongxiang Xu's *in situ* approach showed that he comprehended stem cell activities through the effects of physiological environment. The western research was, on the other hand, limited in the microenvironment of stem cells.

By cloning and purifying nearly 2000 growth factors, *in vitro* stem cell research had succeeded in embryonic stem cell culture *in vitro*. However, to treat diseases with cultured tissues and organs through transplantation, the key was how to direct the proliferation and differentiation of stem cells to produce target cells. Before finding answers for this, other questions were coming.

Each cell of human body came from the same zygote and therefore contained same genes. But the outcome of cell activities was division, which enabled specific function. For example, a cardiac cell behaves like a cardiac cell, and a hepatic cell acts like a hepatic cell. The dividing process was initiated during the fetal formation. Once the cells reached the final stage, it seemed that they could not be changed. Why did cellular activities cause different expression in cells with same genes? The answer was that gene expression was a process in which it was regulated by its own. Theoretically, the differentiation of stem cells was regulated by genes. In other words, it is the genes which decided the cell to develop into heart instead of liver cells. The questions were then how and when the determining gene was activated, when it was shut off, and who regulated it.

A cell is an organism and a gene is a substance. So operating a cell is much more complicated than operating a gene. Nonetheless, the western researchers chose the cell instead of the gene as the next study target, mainly because the stem cell research had yet reached the stage of genetic regulation. Other than this objective and procedural factor, another reason could not be excluded, which was that due to the

inherent defect in genetic techniques, researchers intentionally avoided the issue of genetic regulation. Conducted for years, the gene therapy techniques had encountered great difficulties, which drew people out of the gene fervor. People had to admit that it was extremely difficult to solve or even face the problems with gene therapy, such as: to identify and locate the full gene for specific disease; to induce reprogrammed genes with therapeutic value into human body to enable specific protein expression; and to determine the regulatory sequences and introns in the upstream and downstream of genes etc. All of which were poorly explored with little hope. Somehow, the rise of the study on stem cell treatment represented the impatience for gene therapy and the intention to elude it with an alternative approach for clinical treatment. If stem cell study was founded on genetic regulation, then it would follow the path of gene therapy and depart from the fulfillment of clinical treatment with stem cells. Nevertheless, along with the further exploration in stem cell culture *in vitro*, gene regulation inevitably needed to be addressed and investigated.

At the same time, during the *in vitro* study, researchers had realized that the environment had major effects on stem cell activities. They recognized that stem cell regulation was performed not only by gene, but also by environment, and the latter could even be the determine factor. However, in contrast to Rongxiang Xu, who defined physiological environment as stem cell regulatory environment, western researchers' recognition on environment was limited in 'microenvironment'. The summary article '*Stem Cells: A New Lease on Life*' published in the journal of 'Cell' in January systematically presented the understanding of western scientists and researchers on microenvironment.

'Ecological niche' was believed to be the microenvironment in which cells resided. Western researchers firstly raised the following questions: □What is provided by ecological niche?□ and □How important the ecological niche is?□ They deduced through analysis and review of study results: stem cells in adult human normally reside in specific niche whose microenvironment produces internal or external signals that partly contribute to the determination of destination of stem cells. It is important, among the key environmental factors that decide the differentiation process and activities of stem cells, to find and analyze stem cell ecological niche, and to identify the molecules responsible for regulating specific developmental procedures.

The scientists were also positive about the decisive effects of the ecological niche microenvironment on the generation and differentiation of stem cells. They stated: now we have to face the fact that the higher vertebrates contain lots of ecological niches. They contribute to the maintenance and self renewal of stem cells, and determine the destination of stem cells. Environment or ecological niche decisively, but not necessarily unchangeably, influences the ability of stem cells to choose their fate and destination. In stem cell biology, the most important challenge concerning deciding the fate of stem cells in their embryonic counterparts is to define the key composition of ecological niche, which affects many features expressed by stem cells in their residing niches.

However, some recent experimental results made scientists confused about the

environmental effects, because they showed that stem cells were more dependent on environment, i.e. hematopoietic stem cells coming from bone marrow in an adult mouse were injected into the inner cell mass of mouse embryonic cells, and each step in the following hematopoietic process was tracked. These adult cells were reprocessed to express fetal globulin genes! But interestingly, when the fetal hematopoietic stem cells were injected into an adult spleen, they acted like adult maternal cells and expressed adult globulin genes.

The expressed dependence of stem cells on environment seemed inconsistent with what western researchers had originally believed. Stem cells were divided by previously established procedures. There seemed no necessary correlation between their dividing patterns and the microenvironment in the ecological niches; in other words, it was highly likely that once the stem cells, which were suppose to proliferate and form liver, entered into a certain environment, they mutated to express other tissue cells instead of forming liver! Stem cells were affected by the environment so heavily that it completely exceeded the ecological niche concept defined by western researchers. It also seriously challenged the attempt to build a one-to-one correlation between stem cell expression and ecological niche. These researchers started to question the genuineness of the existence of immature, undifferentiated stem cells. There was an irresolvable contradiction: on one hand, if embryonic stem cells were indeed characterized by undifferentiated state, why instead of differentiating and expressing certain features as originally designed, they were greatly affected by environment and changed freely like chameleon?! On the other hand, if the determine effect of environment was an undeniable fact, people have to ask: 'Do stem cells have the assumed feature of directional differentiation' This would be a dangerous or disastrous question. If the primitive undifferentiated state of stem cells was rejected, what was the foundation of *in vitro* stem cell tissue engineering and regeneration study previously based on it? Could directed differentiation based on it from *in vitro* stem cell culture be accomplished? Could *in vitro* stem cell culture, greatly expected by people, still survive? Probably because this discussion would decide the survival of stem cell tissue engineering, the great project of the century worldwide, scientists affectingly declared: 'Emotionally, we have to face the fact' ecological niche, under certain conditions greatly, affect the morphology and biochemical features of stem cells, so that the superficial behaviors of certain stem cells make researchers confused and suspect that these cells are not primitive enough to be stem cells, if we can not judge a stem cell by its appearance, then what can we do about stem cells and their differentiation feature? Unless stem cells indeed have potential proliferation and self renewal ability by which they adjust themselves according to the surrounding environment, and can choose specific cell line by the signals sent by ecological niche.'

From the speech above, it was not difficult to notice that western researchers' speculation about the relationship between stem cells and environment went far beyond the microenvironment basis. They even considered bringing in new and broader environmental concepts because the actual effects on stem cells went outside the microenvironment of ecological niche. However, they worried that this would be available only after the current appreciation in the features of stem cell activities was changed, as they said, unless stem cells indeed had potent proliferation and self renewal ability.

The emphasis on microenvironment in ecological niche indicated that western researchers' understanding in stem cell was changed fundamentally. Previously they believed that embryonic stem cells had the property of originally undifferentiated state. They have been trying to identify individual expression function of the more than 100 types of stem cells; they isolated stem cells and *in vitro* regulated and maintained their differentiation and proliferation by adding differentiation and proliferation growth factors, and they believed it could possibly achieve directed differentiation of target tissues by above mentioned methods. Now, they realized that it would not be that simple. The real factor which determines the specialized differentiation is not stem cells themselves, but most likely, the environment where they reside.

Despite the breakthrough improvement of understanding in environmental effects on stem cells among western researchers, they are still on the microenvironment level. Furthermore, their knowledge is based on non-confirmed deduction. There is still no substantial evidence to show that to what extent their opinion reflected the actual activities of stem cells.

Where does *in vitro* stem cell culturing head to and who will be able to show it the direction?

When the scientists around the world are lingering between the history and the future, Rongxiang Xu announced his *in situ* stem cell culture approach, which is the most proper answer for the questions above. His *in situ* approach undoubtedly proved the environmental determinism deduced by western researchers, and at the same time, eliminated their limitation of individual cellular microenvironment. Thus, the environmental determinism was more accurately described on physiological level.

3. In situ approach showed direction and indicated physiological environmental determinism

Through Rongxiang Xu's clinical practice and stem cell experiments, it is not hard to find that the effects of physiological environment on stem cells are crucial. Firstly, the activation and generation of keratin 19 positive stem cells were induced by the special medication, MEBO, there were no stem cells in normal tissues or tissues without application of MEBO; secondly, the differentiation of keratin 19 positive stem cells and the repair of injured skin tissues were also completed under the direct effects of MEBT/MEBO. Experiments showed that simultaneous with the changes of keratin 19 positive cells, including their generation, disappearance, and the increase and decrease of their numbers, keratin 9 and 16 positive cells were also produced, which suggested that the differentiation of stem cells was directly influenced by MEBT/MEBO.

The next question is: the significance of MEBO for stem cells, in other words, how it decisively affected stem cells. By studying the pharmacological structure of MEBO,

it is easy to find out the skin replacement function of MEBO. When covered on the wound, MEBO became ideal skin replacement for wound tissues. Firstly, mediated by its oily substances, MEBO provides moist environment appropriate for tissue growth in the wound; secondly, its oily wax structure provides special dosage form covering the wound, which closes the wound to avoid exposure, and preserves the communication between wound tissues and external environment at the same time; in addition, the medication is designed with bionic principle and provides nutrients essential for stem cells. Particularly, MEBO's three-dimensional composition forms a bridge structure suitable for tissue regeneration in the wound. This was validated by the recently published American literature on three-dimensional structure for *in vitro* skin tissue culture.

The above showed that the design of MEBO was based neither on genetic regulation, nor on regulation by microenvironment in ecological niche in tissue stem cells. MEBO was designed to create the physiological environment of the entire wound. For injured skin, MEBO functioned as a temporary replacement. It contains all functions of normal skin, and provides essential nutrients for skin growth. It also maximizes physiological wound recovery. Only by the physiological environment elaborately created by MEBO, is the life potential of stem cells activated and utilized to the extreme.

The revelation of the decisive effects of physiological environment on stem cells significantly facilitated the re-recognition of stem cells, the re-evaluation of *in vitro* stem cell study, and the creation of brand new medical model.

I. Re-recognition of stem cells. The stem cell activities revealed by *in situ* stem cells enriched the understanding about stem cells. They helped people to find out some features of stem cells previously believed non-existing. Firstly, Rongxiang Xu's stem cell experiments showed an undeniably objective phenomenon, i.e. the original undifferentiated feature of embryonic stem cells was beyond question. Embryonic stem cells were able to undergo specific differentiation and led to tissue repair. Moreover, embryonic stem cells can also be regenerated in adult tissues! The existence of embryonic stem cells in adult tissues was a big discovery. Consistent with certain experiment results obtained by western researchers, it demonstrated the interchangeability of stem cells. Secondly, it demonstrated the inherent regenerative and repair potential of organism, which was activated in the rebuilt physiological environment in injured tissues to produce embryonic stem cells and subsequently to repair the tissues.

Embryonic stem cells with differentiation potential were believed to be found only in embryonic tissues. In adult tissues, because the embryonic stem cells had fully differentiated, it was impossible to find them. However, the above *in situ* stem cell study changed people's mind by the facts. It proved that in the rebuilt physiological environment in injured tissues, embryonic stem cells could be produced in adult tissues and underwent typical differentiation as regulated. The skin repairing embryonic stem cells cultured in adult tissues objectively reflected the exchangeability between cells, which included transforming from undifferentiated

state into specifically differentiated state, or vise versa, from differentiated state to the original undifferentiated state, otherwise the production of embryonic stem cells in adult tissues could not be explained. For this, Rongxiang Xu had acquired clear evidence to support it through his bone drilling treatment in which the bone of a patient with local burn and loss of muscle tissues was drilled. The wound and effused tissues through the drill hole was covered and cultured by MEBO, which resulted in full recovery of muscle, skin, blood vessel, and nerve tissues. This further indicated that in the rebuilt physiological environment, culture of bone marrow produced not only skin embryonic stem cells, but also other necessary tissue embryonic stem cells, such as muscle tissues, blood vessel tissues, and nerve tissues. If the transformation of stem cells was so easy, then it proved the unwilling speculation of western researchers that stem cells were likely to have potent proliferation and self renewal ability.

Some western animal studies offered similar evidences. The recent experiment on mice demonstrated that when put into bone marrow, neural stem cells were able to produce all blood cells; another mice experiment showed that the stem cells found in bone marrow generated liver cells. On October 26th, *American Society of Plastic Surgeons* announced more convincing results. Two research groups found that after adding growth factors which are able to stimulating bone growth into cultured cells, osteocytes were generated by fat stem cells. This finding initiated the attempts to generate muscle by fat stem cells.

Rongxiang Xu's stem cell research revealed the production of keratin 19 positive cells and their natural progress of differentiation and skin repair. Other than the physiological environment provided by MEBO, there were no artificial factors to direct and regulate the differentiation of the stem cells. Regardless of environmental factors, the stem cells acted naturally with dynamic and autonomy, resembling construction contractors following the blueprint and schedules to prepare materials, organize personnel, and conduct construction. The key contribution of stem cells' dynamic role is undoubted; the question is how the stem cells received signals and was activated.

Rongxiang Xu's stem cell research also showed the determine effects of physiological environment, and therefore clarified the understanding about environment. He established the correlation between stem cell activities and the environment. In detail, as organisms, stem cells' activities were closely associated with the entire body. Changes caused by injuries or disorders of certain part were signals themselves, and were transmitted by internal biochemical reaction; these signals initiated induction and differentiation of embryonic stem cells and subsequent tissue regenerative repair, which necessitated the rebuilt normal physiological environment; in normal physiological environment, stem cells acted strictly in accordance with the blueprint acquired from zygote, and repeated the developmental process during early embryonic period. Moreover, by comparing the original embryonic blueprint with current body state, stem cells accurately defined the scale, composition, and amount of tissues needed for tissue regenerative repair.

The rebuilding of physiological environment was essential in the above process. Without physiological environment rebuilding, embryonic stem cells could not be automatically produced in the injured tissues, which indicated that injuries by themselves were insufficient to signal the dynamic reaction in stem cells. Only after physiological environment was rebuilt and with sufficient regenerative substances, did the signals of body defect and repair demanding initiate the potential function of tissue regeneration to induce embryonic stem cells by which the rebuilding and repair of injured body were accomplished.

II. Re-evaluation of *in vitro* stem cell study. The substantial changes in the understanding of stem cells objectively lifted all current stem cell researches onto a new level. The determine effects of physiological environment on stem cells revealed by Rongxiang Xu clarified *in vitro* stem cell research and exposed the potential difficulties underneath.

Physiological environment in the human body determined the initiation of stem cell regeneration and directional differentiation. In other words, regeneration and differentiation of stem cells could not be achieved without the entire human body physiological environment. The life activities of stem cell were unconditionally dependant on the human body's physiological environment. However, the life activities of stem cell were totally contradicted by the *in vitro* stem cell study on this. The techniques adopted by *in vitro* stem cell research maximally separated stem cells with original physiological environment *in vivo*, which was seen throughout the entire process of *in vitro* stem cell culture.

The first step is the isolation of stem cells. In theory, stem cell could be isolated from embryonic tissues or adult tissues. In fact, adult stem cells had not been successfully isolated from each human tissue. The major setback for isolation of stem cells from adult tissues is the difficulties in identifying stem cells, because the morphology of all cells had few differences. Since it was difficult to identify stem cells, it was nearly impossible to isolate them. Although various types of stem cells were identified, stem cells had not been found in all of the tissues in adult human body. For example, adult stem cells in heart and pancreatic islets had not been identified yet. In addition, the limited number of adult stem cells made the isolation and purification more difficult. The alternative pathway of isolating stem cell from embryonic tissues was feasible and relatively unproblematic, except that the directed differentiation through *in vitro* culture was still challenging.

After isolation, both embryonic and adult stem cells lost their connection with the organism. When within the body, the life activities of stem cells, including the activation, termination, and direction of differentiation were organically consistent with, and directly influenced by whole body environment, in other words, the signals activating the directed differentiation of stem cells came from the collaborative development of the entire body. Once stem cells were separated from the body, the transmission of signals essential for their life activities was stopped. Consequently, without the control and commend from the body, the growth and development of stem cells were dependant on the artificial environment *in vitro*. The question was, can

stem cells survive and continue the specific differentiation in artificial environment? Current researches showed that stem cells could survive and proliferate *in vitro*. However, by the revelation of its effects on stem cells, the physiological environment was the primary issue in the directed differentiation of stem cells *in vitro*.

Could directed differentiation be accomplished in the environment provided by *in vitro* stem cell culture? This should be addressed by *in vitro* stem cell research, because it was associated with the foundation of the *in vitro* stem cell culture research. If the answer is 'YES': the isolated stem cells, under artificial regulation, could directionally differentiate without original physiological environment, then *in vitro* stem cell culture would be a realistic goal worth to be dedicatedly explored further; on the other hand, if the above situation was not the case, then *in vitro* stem cell culture research would be an illusion at most, despite their attractive prospect.

The second step is *in vitro* stem cell culturing, which is also the most challenging part in the research. The key for *in vitro* culture is to provide appropriate condition for stem cell growth and development. The condition involves not only nutrition, which would be easily regulated, but also the signals from the body, which made *in vitro* culture complicated. It is still poorly understood on what condition is suitable for stem cell growth and development, and the effects on initiation, proliferation, differentiation, and termination of stem cells even *in vivo*, let alone *in vitro*. Therefore, there is likely an impassable gap between the understanding of environment effects on stem cells and the current research on *in vitro* stem cell culturing.

Currently, *in vitro* stem cell culture research focuses on exterior tissue supporting materials and cell growth factors that controlled the cell division. Most artificial tissue supporter is made of bone collagen sponge. Bone collagen can be dissolved *in vivo* and absorbed by tissues in the process of tissue metabolism. Cell growth factors were regenerative proteins presumably able to initiate cell proliferation, differentiation, and regeneration, which were poorly understood. The artificial environment of *in vitro* stem cell culture is consisted of only the above two factors and therefore, is significantly different to the *in vivo* physiological environment essential for stem cells. If the artificial environment is evaluated by the restored physiological environment of the organism, then the microcosmic restoration of the environment would be extremely difficult to succeed. By the improvement of understanding of life activities on molecular level, it would be accomplished in the future without doubt, but it would only be accomplished on the premise that every detail in life activities is analyzed and understood on molecular level.

The most important difference between *in vitro* and *in situ* culturing is the dependence of stem cells on the organism. *In vitro* culture is based on the knowledge that stem cells could differentiate and proliferate *in vitro* without the organism. In other words, the state of body was insignificant in the process of culturing. Its significance was considered only at the tissue or organ transplantation stage when the immune rejection of cultured tissues or organs by the host needed to be addressed.

There is no evidence from *in vitro* culture study showing that the immune rejection of

cultured tissues or organs could be avoided. Some researchers assume by tissue homogenicity that *in vitro* cultured tissues or organs should not be rejected. While, other researchers believe that tissues or organs cultured by embryonic cells are different to adult tissues and therefore the possibility of rejection could not be ignored. Tissues or organs cultured from adult tissue cells might not cause rejection, but there are other difficulties such as identification and isolation of adult stem cells. In addition, the possibility of mutation of adult stem cells caused by environmental pressure increases the risk of genetic defects.

The decisive effects of physiological environment on stem cells revealed by Rongxiang Xu were constant instead of paroxysmal throughout the whole process. Even if tissues or organs could be cultured *in vitro*, the combination of them and the body would not be restored to the original state of body tissues. During the process of body repair, tissues and organs cultured *in vitro* obtained certain unalterable features from the outside, which have been demonstrated by some studies. For example, scientists treated severe burn patients with *in vitro* cultured dermis and epidermis complex, but still resulted in scar healing. The wound could not be restored to the original state naturally developed from maternal birth. This indicated that without original environment, stem cell expression was inconsistent with the organic system. The consistence could not be simply created through the control of rejection. Instead, it needed not environmental regulation at certain stage and time, but the intricate organic signals during the entire process of stem cell generation and division to establish the consistence.

III. Establishment of brand new medical model. _The value of western stem cell research was based on clinical treatment, so was Rongxiang Xu's research. However, there was difference between them. The western stem cell therapeutics was based on the already established tissue engineering in which stem cell research must follow the overall purpose of tissue engineering. Therefore, stem cell, as positioned by tissue engineering, is to provide supplies for tissue and organ transplantation, which greatly limited stem cell research and obscured its great potential. This is the reason people believed that the value of stem cell research did not go beyond tissue engineering. In fact, tissue engineering became the reference system of stem cell research.

Rongxiang Xu's successful *in situ* stem cell culturing revealed that stem cells' activities went far beyond people's imagination. Embryonic stem cells not only have the potential to differentiate into specific target tissues, under the restored physiological environment with sufficient nutrients, they are able to be induced and differentiated in adult tissues, and then complete the tissue regenerative repair. If mature adult stem cells could be transformed into embryonic stem cells, then the main focus in tissue culturing should be to encourage the induction and transformation of mature stem cells into embryonic stem cells without the need to proliferate embryonic stem cells *in vitro*. This provided great potential and sufficient supplies of stem cells for tissue regeneration. Then what is the practical meaning of *in vitro* artificial stem cell culturing?

In situ stem cell culturing highlighted the clinical value of stem cell therapeutics.

Stem cell treatment should not be simply the supplies of tissue and organ transplantation. People who were excited about the minor body mending by stem cells really underestimated the potential of stem cell, the seed of life. Stem cells contribute to human medicine by regenerative repair, which was fully demonstrated by Rongxiang Xu. As described above, regenerative repair can achieve not only physiological healing of injured skin tissues without scar, but also the synchronic, holistic, and physiological repair in various tissues, such as skin, blood vessel, and nerve.

Regenerative repair are not simply referring to change the diseased tissues or organs, it is to completely normalize the diseased tissues and restore their original state. This therapeutic purpose had never been imagined before. It would lift clinical medicine on an unknown whole new level. And this is a truly exciting goal. Despite that there might still be a long way to go before getting to the destination, Rongxiang Xu's effective work in skin repair made it far more reachable than stem cell tissue engineering, which is hopelessly far away from any clinical achievement. Regenerative repair is not a dream anymore, it is a real existence.

[Life is not yet a machine which can be disassembled freely.]

In stem cell research, the difference between *in vitro* and *in vivo* mode reflected not only the different understanding between western and eastern researchers on stem cell activities, but also the difference on the in depth recognition on the nature of life. Inspired by molecular biology, *in vitro* researchers observed life activities with a mechanistic point of view, which had dominated the understanding of life since 20th century; the success of Rongxiang Xu's MEBT techniques and the emergence of *in situ* stem cell culture slightly retrogressed people's understanding on life nature from today back to the history. When the whole world is mesmerizing in the hope of revealing the mystery of life, and extremely appraising people's recognition ability, Rongxiang Xu showed the world the other side potential of life itself. This revelation is not merely a demonstration. It offered an implicational feeling to the people as God given. Rongxiang Xu, at the frontline, in the research field in which human power was expressed to the extreme, at the most imaginative and proudest moment, with his ancient eastern intelligence fulfilled the unreachable dream which could not be fulfilled even with all human intelligence combined. It is incredible, like God was joking and playing the cat and mouse with human being. What is the meaning behind Rongxiang Xu and his research results? Is there a supernatural power manipulating its creation, challenging human's intelligence, and sneering at the human vulnerability?

In 1912, Germany born biologist Loeb published his epoch-making book, '*The Mechanistic Conception of Life*' when migrating to America. In the book he described his own experiment on the eggs of sea urchin. Loeb took the eggs out of female sea urchin and without sperm, used chemicals to stimulate female urchin to mimic fertilization, and therefore produced the most surprising phenomenon the generation of an 'embryo'. Although today this experiment becomes a simple demonstration to show the early development of embryo, back to that time, this process of monocellular development into organism was described on newspaper headline as production of life

in the test tube. Loeb's study and discovery became the clear evidence to support the mechanistic conception of life. He truly believed that the mechanisms of life were as easy as that life could be produced in laboratory. He wrote: □We will successfully produce artificial life; even not, we will find the reason.□

Since 1970s, the research in life activities had been carried out on the cell and molecule combination level to explore the nature of life with mechanistic approach. Today, some life activities can be performed without organism, and the fundamental process of life could be explained by the interaction between nonliving atoms and molecules. Contemporary biologists claimed: □All life building phenomena can be explained by physics and chemistry vocabularies.□

By the success of life cloning from somatic cells and indefinite proliferation through *in vitro* stem cell culture, mechanistic understanding of life was pushed to the extreme. It was reported that the study groups in American and Canada had announced to initiate an ambitious plan with 3 billion British pounds of budget to produce human organs. They would clone the various tissues of the organ first, and then assemble the tissues to form the organ. For example, to produce a heart, firstly they would use stem cells from different tissues to produce cardiac muscle, heart valve, and blood vessel tissues, and then put these tissues together to form a heart. The target organs included heart, kidney, and liver, etc.

In history, although some western scientists realized the 'self constitution' ability of life, it was used, instead of supporting organicism, only for describing life dynamics which could not be explained by their mechanisticism. When facing life dynamics, their explanation was that it was unnecessary to explain it, like the formation of snow flake, which was not by miracle. The snow flake crystallization was originated by some specific features of molecules. It was self constitution without influence of external factors. Specific molecules could form specific structures because their physical and chemical properties only allow them to form those structures. In other words, the crystallization was caused by the internal factors inside the molecules without the influence of external factors. The French Nobel Prize winning biologist Monod wrote in his book '*Chance and Necessity*': □Pre-constituted complete structures do not exist, but their blueprints are stored in each constitution unit, thus, without external help or inserted extra information, they can be formed automatically and spontaneously. The essential information is partially concealed in the forming molecules, and a structure is formed gradually instead of suddenly from nothing. It is the demonstration of the message.□

From global to local, from macrocosmic to microcosmic, the understanding on life nature was gradually expanded. It was fundamentally improved from the superficial level into the intrinsic level. The life nature was successively revealed on organism system organ tissue cell level. The understanding was guided further into microcosmic level on which people was inspired by the discovery of 'life mechanistic' details without noticing the macrocosmic level, let alone the inevitable correlation between microcosmic and macrocosmic level. It seemed that all of the significance of

life activities lay on the microcosmic level and in the cells; even the occasional consideration about the association between molecules and external environment was focused on microenvironment.

Microcosmically, organism is a macro-system. Its life activities are complicatedly and uncontrollably presented on different levels. It would be difficult even to thoroughly understand the activities of cell, the most fundamental unit of organism. To investigate life on cellular level, both internal and external constitution must be clarified. As pointed out by the scientists, the blueprint was in the forming molecules. However, what if was the blueprint not only in the molecules, but also outside the molecules, or even in the interaction between molecules? In this case, identification of life activities would not be simple and accomplished by the understanding only on the local features of organism.

According to life mechanisticism, life is as simple as a computer. No matter how complicated are the circuits, integrated circuit could participate in the computer formation as an independent unit which can be changed with a new one and still work as usual. Is this the case in the constitution of real life? 2000 years ago, Aristotle from ancient Greece used an oldest metaphor to describe the local and global relationship: hand was connected with arm. When form as a united organism, hand functioned as hand. When separated from arm and lost the unison with the organism, hand lost its function and was not hand any more, even it still kept the appearance. This was the most original and plain organic theory.

As early as in 1930s, physiologist Holden criticized that the pure mechanistic explanation could not clarify the phenomenon of life unison. Ritter invented the word 'organicism' to demonstrate the organic theory of life. He described life as: the correlation between the entirety and the components, including the sequential regulation between components and their interdependence essential for the existence of the entirety, as well as the absolute control of the components by the entirety. Novikoff showed in detail that why the explanation of organism must be integrative: The whole on a certain level is the component of a higher level. Both the parts and the wholes are substantial entities, and the integration caused by the interaction between components is resulted from the integrative feature of the components. Because the vitalism excludes the reductionism, it opposed to describe the organism as a machine consisted of independent parts (physical and chemical units), which could be removed like taking a piston from the engine, and all parts could be described by their functions and natures without mentioning their origins. In contrast, due to the interaction between the components in biological system, the merely description of each component was unable to demonstrate the integrative nature of the system. It was the tissue between the components actually controlled the entire system.

From cell to tissue, organ, organ system, and the entire organism, there is component integration on each level, which can be seen on biochemical level, genetic level, and individual level. All organicists agree that no system can be clearly explained by the natures of each component. Organicism is based on the fact that organism contains

tissues. Organism is not formed as a pile of stones by properties and molecules. The function of organisms is determined by properties, intermolecular tissues, intermolecular correlation, molecular interaction, and molecular interdependence.

The advanced development in microcosmic study in life led people to thorough understanding and away from entirety at the same time. It seemed difficult for people to globally or systematically view life. Under this background, Rongxiang Xu's *in situ* stem cell research led people back towards the history and the recognition of life with systemic organic point of view.

The correlation between stem cells and organic physiological environment established by Rongxiang Xu revealed that the significance of life was not only organic, but also dynamic. In other words, on one hand, life is a united and integrative system in which each part is controlled by the organism to meet its global needs and can not independently exist. Without the connection with the organism, each part would lose its specific function in specific organism and therefore, lose its meaning. On the other hand, life is a dynamic system in which each part positions itself and performs its own tasks in the united environment and under the commend from the organism in order to support the whole organism.

Because its simplicity and primitiveness, the organic view establishes a straightforward and principled concept about life activities. No matter how thorough and deep the microcosmic understanding about life is, even on the molecular level to study the basic unit of cell and its fertilization, differentiation, growth and proliferation, and signal transmission, it should not contradict with the fundamental characteristics of life, which are the organic nature of cell activities revealed, on the basis of realistic clinical achievements, by Rongxiang Xu's stem cell research.

Based on the activation of somatic cells to become stem cells, and on the directed differentiation of *in situ* stem cells, let's review the stem cell activities: when skin was injured, it was managed by Rongxiang Xu in whole body macrocosmic physiological environment, instead on microcosmic molecular level, which maximally and artificially built the physiological environment for the wound; under the condition by which the basic life activities were functionally maintained, the organism automatically sent signals through information exchange in order to repair the injured tissues; provided with bionic designed essential regenerative nutrients, somatic cells in the organism automatically initiated the procedures directed by the commend and transformed into primitive embryonic stem cells; the embryonic stem cells then started tissue regeneration by the organic differentiation procedures acquired during the zygote period. However, during the process of tissue regeneration, embryonic stem cells did not passively produce the tissues as the origin. They accurately determined the amount of repairing tissues by the severity of the injury, and therefore to produce optimal healing without excessive proliferation which might cause skin protrusion, or inadequate repair which might lead to sunken skin. This is the true dynamic feature of stem cell life activities disclosed by Rongxiang Xu.

Facing the perfectly repaired muscle and skin, we have to wonder: in this world, what kind of stem cells can accomplish this marvelous masterpiece as *in situ* stem cells, and what kind of ability can exceed the great potential hidden in life itself.

Finally, we come back again to the old issue pursued by philosophers for thousands of years, which is the relationship between human and nature. In front of the objective world, how powerful is human dynamics? Can human being change the natural progress of life on the most fundamental level and therefore to beat the nature? If we can serve people by conforming to the nature, then what is the point to exhaustedly struggle with and finally to go beyond the nature? Back to the reality, what we consider will be more practical. If life tissues can restore the physiological environment for diseased organisms and provide the prerequisite activation conditions for stem cells with low costs, and therefore to achieve the regeneration and repair of organic tissues, then what is the practical value of the tissue engineering involving *in vitro* tissue culturing and organ transplantation by stem cell?

[Closing remarks]

In contrast to his luck on scientific study, Rongxiang Xu's life experience was full of frustrations. He founded MEBT techniques and brought burn medicine in a new era, however, he was misunderstood, criticized, and blamed for no reason at the same time; he invented bionic medication to save millions of lives from suffering, and was forced to struggle with traditional opinions to obtain registration permit; he raised the most advanced scientific proposal and proposed the molecular medicine journey with a capable team, and what will be waiting for him?

Rongxiang Xu's adversity may be caused by the fact that his scientific beliefs and activities are too advanced to be accepted. Even if one has the truth, it still takes a long while for the truth to be accepted by the tradition and public. The good thing is: truth is truth nonetheless and time is the best touchstone. No matter how long the waiting, how huge the setback, time will not cover the truth; it will only make it dazzling bright.

The MEBT techniques brought burn medicine into a new era worldwide. Now the study results of *in situ* stem cell culture obtained by Rongxiang Xu will lead the world into a new era of regeneration never seen before.