Minutes of the Third ABCC/RERF History Forum

Speaker: Dr. Akio Awa, former Associate Chief of Research and Chief of Department of Genetics

Host: Mr. Takanobu Teramoto, Executive Director

Date: 15:00 - 16:10, October 10 (Fri.), 2014

Place: Videoconference: Auditorium in Hiroshima Laboratory and Third-floor Conference Room in Nagasaki Laboratory

(Honorifics omitted)

Katayama (Chief of Department of Information Technology): We invited Dr. Akio Awa, a consultant to the Department of Genetics and former Chief of Department of Genetics and Associate Chief of Research, as the speaker for this history forum. Mr. Teramoto will serve as the host.

Teramoto: Thank you for your introduction, Dr. Katayama. This is the third history forum. The previous two speakers were general employees at ABCC/RERF in Nagasaki and Hiroshima, and the minutes of these two forums are posted on the RERF official website. The speaker today is a retired researcher, Dr. Awa.

In 1967, Dr. Awa graduated from the Department of Zoology, Hokkaido University School of Science and joined ABCC. He worked at ABCC for eight years from 1967 until ABCC's reorganization into RERF in 1975 and at RERF for about 20 years until his retirement. He was appointed the first chief of the Department of Genetics in 1985, promoted to Associate Chief of Research in 1994, and retired when he reached the mandatory retirement age in 1995. He then served as an RERF consultant for the next four years, the last two years of which he concurrently served as Associate Chief of Research again. He is now serving as a consultant to the Department of Genetics.

Dr. Awa, you contributed an article titled, "Ten Thousand Days Atop Hijiyama" to the RERF *Update*, which was published in its Spring 2002 and Spring 2003 issues. The article summarized the history of your academic life since you joined ABCC, dedicated to the work of developing a technology for estimating radiation doses based on chromosome aberration. I assume this will be the main topic of your talk today. Would you first tell us how you joined ABCC?

Awa: Thank you for your introduction. I spent two years at the University of Texas Medical Branch at Galveston and later in Pasadena, California between 1960 and 1962. I was in my thirties then, searching for a direction in my research career, and I chose the field of chromosome research.

Teramoto: You then decided to implement chromosome studies at ABCC?

Awa: Yes. Dr. Michael Bender* of the Oak Ridge National Laboratory (ORNL) came to Sapporo in 1963 and invited me to work at ABCC, which needed to initiate chromosome research. At that time, I had a Ph.D., but was working at Hokkaido University with no pay,

making a living doing part-time jobs. One year after I received the job offer from Dr. Bender, I had an opportunity to talk with Dr. Ernest Chu of the ORNL in Ohdori Park in Sapporo. He also encouraged me to work at ABCC. He seemed to be a cheerful and sincere person, so I thought that it would not be a bad idea to work at ABCC, recommended by such a good person. At least it would be better than working for a hardheaded professor at the university. Although it is not nice to speak ill of the deceased, he really was an intimidating professor.

Teramoto: You said you chose chromosome research while you were working at the university. Was a significant advance made in the field of chromosome or DNA research around that time in the 1950s?

Awa: Watson and Crick discovered the structure of DNA in 1953. Shortly after that, it was proven that human beings have 46 chromosomes, not 48. It became possible to visualize chromosomes in cultured blood lymphocytes and analyze them.

Teramoto: After you joined ABCC, you initiated your work of chromosome analyses. Were you off to a good start?

Awa: What bothered me the most was the scarcity of microscopes. Each worker needed one dedicated microscope. To observe chromosomes, they need to be magnified 1,000 times. However, the largest chromosome is 19 μm, and 1,000-fold magnification would not make them too easy to observe. Our job was to take photographs of chromosomes through microscopes, then sketch and analyze them. It was the dawn of the age of full-scale chromosome research. My work started from scratch, and researchers were competing in human chromosome research. Microscopes were the most important item for researchers who were studying chromosomes. Thus, we requested that microscopes, however expensive, be purchased.

Teramoto: You investigated the frequency of chromosome aberrations. I understand that some types of aberrations are very short lived (unstable chromosome aberrations) and some others are persistent for decades (stable chromosome aberrations). Did you wonder which type you should focus on?

Awa: It is easy to detect chromosome aberrations immediately after radiation exposure. However, I joined ABCC more than 20 years after the atomic bombings. I was absolutely convinced from my work experience in the U.S. that A-bomb effects had been engraved in chromosomes as aberrations, so I was anticipating various positive results even before we initiated our studies.

When blood samples collected from human beings are cultured, only lymphocytes survive. If we prepare samples exactly when the surviving lymphocytes start to undergo the first cell division, we will have beautiful chromosome samples, which enable us to identify individuals with damaged DNA. I was convinced that the technology I learned in the U.S. could be utilized at ABCC.

Teramoto: That is probably why you pursued the study of stable-type aberrations with dedication. What was your first impression of Hiroshima?

Awa: The first two days after I arrived in Hiroshima were very clear and sunny. It was January.

I stayed at Hijiyama Hall and looked for an apartment until my family joined me in Hiroshima. Hijiyama Hall was called the BOQ, short for "Bachelors Officers Quarters" back then and food was served there. Dr. George B. Darling, then ABCC Director, and ABCC researchers lived in the BOQ.

Teramoto: You continued your studies of chromosome aberrations, which led to the finding that chromosome aberration frequencies were in proportion to the T65D dose estimates. Meanwhile, cases with a discrepancy between chromosome aberration frequencies and T65D dose estimates were observed, and researchers tried to determine whether the discrepancy stemmed from errors in dosimetry or from biological individual differences. This story is described in your article in the *Update*. Could you share with us some anecdotes about your struggles?

Awa: We found several unbelievable cases while examining the relationship between dose estimates and chromosome aberration frequencies. They included proximally-exposed survivors with no chromosome aberrations and distally-exposed survivors with chromosome aberrations. We called them DCs (discrepancy cases), cases with a discrepancy from prediction. I assume those cases included individuals who wanted to hide the fact that they had been exposed to A-bomb radiation for marriage or various other reasons.

Teramoto: Was there a dispute between the Departments of Statistics and Genetics over the interpretation of this discrepancy?

Awa: Yes, and each group refused to yield.

Teramoto: By that time, the biennial blood sample collection from AHS participants had already begun. I assume researchers examined samples collected from the same subjects on multiple occasions and concluded that the argument of your group was correct.

Awa: Yes. We examined each sample many times, in some cases as many as 10 times, and determined that no individual variation was involved. However, what troubled us was that chromosome aberrations might increase with age. Dying lymphocytes in particular have an enormous number of aberrations. I called such lymphocytes "swan-song lymphocytes." Lymphocytes die after completing their jobs of ingesting bacteria and other things. I do not remember lymphocytes' lifespan. [*An attendee said it is two to three years.*] Thymic lymphocytes live quite long. I hypothesized that chromosome aberrations deteriorate DNA functions, resulting in premature death. However, no study has ever been conducted to examine individual thymic lymphocyte. So this is my personal hypothesis.

Okubo: Each living organism has both dying and new lymphocytes. Will the age of the lymphocyte examined affect the result of examination?

Awa: The issue of lymphocytes' lifespan is difficult. Lymphocytes die for various reasons. Some die after ingesting invaders that they encounter, while new ones enter the circulation one after another. Yet, for example, some individuals continue to have chromosome aberrations 40 years after their A-bomb exposure. I examined chromosomes of A-bomb survivors for 20 years, that is, 10 AHS examination cycles, expecting to see decreased frequencies of aberration cells, but I did not observe any such decrease. I cannot help

wondering why. Another finding that I did not understand was why there were no individual differences. All cases were somehow similar.

Teramoto: Did you examine a certain number of lymphocytes to determine a general trend?

Okubo: Dr. Awa explained to us the changes observed when a given individual was followed up for a certain period of time. Mr. Teramoto's question concerns the variability among 10 lymphocytes, for example.

Awa: We usually examined 100 lymphocytes because it would be easier to calculate percentages.

Okubo: What was the variability among the 100 lymphocytes you examined per one blood sample?

Awa: There was a certain level of variability, but Dr. Dale Preston (former Chief of Department of Statistics), who performed the statistical analysis, said that it was within the range of error.

Teramoto: You continued your studies of chromosome aberrations and developed a biodosimetry that is based on blood samples. Now, could you tell us about any persons or incidents that left a strong impression?

Awa: Dr. Charles Marc Pomerat, my mentor at the University of Texas Medical Branch, left the strongest impression. He served as the chairman of the American Tissue Culture Association. He was usually very good-natured, and we could tell his mood by the way he spoke. He was in a good mood when he spoke fast and in a bad mood when he spoke slowly. What he taught me was very valuable. Without it, I would not have been able to do research at ABCC.

Teramoto: Who left a strong impression at ABCC/RERF?

Awa: There were many such individuals. I am particularly grateful to the heads of the Department of Medicine. Blood drawing was not a common practice in Japan before World War II, but it was in the U.S. There were such differences in culture and customs between the two countries. I think one of the reasons why A-bomb survivors were very reluctant to come to ABCC for health examinations was that they had to give blood for various tests.

Teramoto: This will be my last question. Radiation exposure has become a significant social issue since the Fukushima nuclear power plant accident in 2011. Health surveys of residents of Fukushima prefecture are being conducted, in which RERF researchers are cooperating. Detailed surveys and interviews are needed there, but I assume it is quite difficult to gain the understanding of the human subjects and participants in conducting surveys and studies. Fortunately, as high as 70-80% of the cohort members have participated in our AHS program, and its study results are being used as the basis of international radiation protection standards. I think the AHS can be regarded as a success. I am sure that researchers, technicians and clerical employees went through enormous troubles before the AHS reached that stage. Could you share with us some of your experiences or struggles?

Awa: I gave advice to Fukushima Medical University, which planned to establish a chromosome laboratory after the nuclear power plant accident. It is possible to have the same slides examined by multiple institutions for better accuracy and data.

Y. Kodama: We also had discussions with the university on our possible assistance to them in their efforts of establishing the lab, such as for the training of technicians.

Teramoto: The floor is now open for questions.

Okubo: ABCC/RERF developed physical dosimetry systems, from T65D to DS86 and DS02, while you worked on chromosome-based biodosimetry from 1967. How did your work relate to the development of physical dosimetry? How was the chromosome-based biodosimetry work taken or viewed at RERF?

Awa: Our research is nothing but biodosimetry and will be all right as long as we collaborate with statisticians. Dr. Preston of the Department of Statistics analyzed data for all of my chromosome studies. The plan was to follow up the same A-bomb survivors from different angles.

Okubo: I, as the RERF Chairman, am curious to know whether or not ABCC/RERF appropriated enough budgets for your research.

Awa: I do not have an answer to that question. My studies required monies only for microscopes and photographs. Microscope slides were very cheap. Culture solutions were probably a little more expensive, although they were inexpensive considering the nutrients they contained. I am tone-deaf when it comes to money. Please ask that question to the Accounting Section. [*An attendee said Dr. Awa's studies cost little.*]

Okubo: I just wanted to know how you thought you were regarded.

Awa: I think I am impudent.

By the way, ABCC had a mortuary. Bodies were brought to ABCC during the night, autopsied, examined, and sewn up. Then they were returned to their surviving families after prayers suiting their religions were offered. The staff made such efforts to soothe the resentment of the citizens at ABCC, an American research institute.

Teramoto: That has reminded me of an anecdote in your *Update* article that the Crow Committee's recommendations enabled your studies to continue when there was a dispute over discrepancies between T65D dose estimates and chromosome aberration data.

Awa: The Crow Committee painstakingly examined our data in detail, and showed a future direction for ABCC/RERF research. The committee said that the cytogenetic approach was very effective, which overjoyed me.

Teramoto: The Crow Committee's recommendations turned the tables?

Awa: Not exactly. Dr. Crow made two points. One was that the cytogenetic studies were

conducted only by Japanese researchers while the majority of researchers at ABCC were Americans. The other was that biological dosimetry was a very promising effective approach.

Okubo: Staining methods have changed. I do not believe I can detect chromosome aberrations stained in the old methods, but I feel I can detect aberrations stained in modern methods. Would you tell us when and how the modern methods were developed and the struggles that you had.

Awa: The FISH (fluorescence in situ hybridization) method visualizes translocations, in which parts of different chromosomes are exchanged and recombined. This technology enables even laypersons to detect translocations, each of which is stained in two different colors. Another staining method is the Giemsa banding technique. Combining two or more staining methods will not only make detection of aberrations more accurate but also make it possible to detect new types of aberration.

In 2003, I spent half a year in Oak Ridge to develop a system to be used as a standard, and investigated whether or not the same slides examined in various research institutes in Japan, the U.S., Germany, and France would yield the same results.

Teramoto: The introduction of the FISH technique, which was developed at the Lawrence Livermore National Laboratory (LLNL) and introduced to ABCC in close collaboration with them, is described in the *Update* article.

Awa: When Dr. Mortimer Mendelsohn was an RERF director, I went to the LLNL and conducted studies using various assay systems. We compared assay systems using the same samples.

Teramoto: Thank you for your talk, Dr. Awa. I wish you the best of health and hope that you continue to come to RERF to provide research guidance and advice to our junior investigators.

Katayama: Dr. Awa, thank you for coming and sharing your interesting stories with us.

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