Molecular genetics of cancer: a brief history and my discoveries

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Introduction

As a geneticist in Thailand, I received two unique opportunities. First, I have been appointed to be a scientific advisory committee (SAC) member of Prince Mahidol Award since 2001. Every year, his majesty the king grants the award to outstanding scientists in the field of medicine and public health. As a SAC member, I learnt how a scientist or a group of scientists could contribute for the benefit of mankind. The first part, “A brief history of molecular genetics of cancer”, of this article is a review that regarded the contribution of Professor Axel Ullrich, the 2007 Prince Mahidol Award Laureate.

Last year, I was awarded “Outstanding Scientist Award” by “Foundation for Promotion of Science and Technology under the Patronage of H.M. the King”. Even though doing advance molecular research in Thailand is difficult due to limited resources, we were able to report several unprecedented molecular genetic events. The later part of this chapter is the review of methods that I approached for new discoveries.

A brief history of molecular genetics of cancer

Better understanding in molecular biology of cancer has saved thousands of life and will save millions more. Knowledge in molecular biology has led to the development of a number of useful diagnostic tools for many types of cancers. Examples of these include the detections of Philadelphia chromosome for chronic myelogenous leukemia (CML) diagnostics and acute lymphoblastic leukemia (ALL) prognostic as well as treatment monitoring (Kurzrock, Kantarjian, Druker, & Talpaz, 2003); loss of heterozygosity (LOH) of chromosome 1, 17, and 18 as neuroblastoma (Attiyeh et al., 2005) and colon cancer (Chang, Lin, Lin, & Liang, 2005) prognostic markers; and HER2NEU amplification as breast cancer prognostic markers (Wong et al., 1998). Molecular diagnostic tools have enormous

potentials, especially in cancer screening (Sidransky et al., 1992) and treatment monitoring (Lo et al., 1999). The discovery of etiological factor in cancer such as human papillomavirus high risk group as a cause of cervical cancer (Gissmann, Pfister, & Zur Hausen, 1977; zur Hausen, 2009) by Professor Harald Zur Hausen, the Prince Mahidol Award and Nobel Laureate led to success in using viral DNA as tumor markers (Mutirangura, 2001) for cervical cancer screening (Growdon & Del Carmen, 2008; Kiatpongsan et al., 2006) and nasopharyngeal carcinoma monitoring (Lo et al., 1999; Mutirangura, Pornthanakasem, Sriuranpong, Supiyaphun, & Voravud, 1998a). This suggests that improving molecular (Sidransky, 2002) and nanomedical technologies (Grodzinski, Silver, & Molnar, 2006) will undoubtedly bring about the promising future benefit of this field of medicine. Moreover, as shown by recent examples, the knowledge in this area can also lead to the ultimate goal of cancer cure. Scientists have discovered molecular targets specific to cancer cells and engineered drugs that can act specifically at the targets (Alvarez, Kantarjian, & Cortes, 2007; Carneiro, Hsiao, & Khandekar, 2005; Haber et al., 2005; Jones & Saha, 2005; Ross et al., 2004; Sharkey & Goldenberg, 2006). Consequently, this way of targeted cancer therapy offers better efficacy in killing cancer cells while does no harm to normal cells. Several such oncogenic protein targeted therapies are currently in clinical trials. Herceptin (Trastuzumab) and Gleevec (Imatinib Mesylate) specifically target HER2NEU in breast cancer and BCR-ABL, a chimeric protein product from Philadelphia chromosome, respectively, are among the prototypes that are already in use in current clinical practice (Fischer, Streit, Hart, & Ullrich, 2003; Ross et al., 2004).

To achieve the goal of better cancer diagnosis and treatment, a countless number of scientists and enormous amount of efforts are required. These efforts have led to several important scientific breakthroughs that directed the current stage of achievement. The first was the principle of mutations in the origin of cancers (Cahill, Kinzler, Vogelstein, & Lengauer, 1999; Knudson, 1995; Lengauer, Kinzler, & Vogelstein, 1998; Liu & Weissman, 1992; Nowell, 2002; Varmus, 1984; Vogelstein & Kinzler, 1993). Second was the identification of oncogenes (Varmus, 1984), tumor suppressor genes (Carbone & Minna, 1993; Knudson, 1993) and down-stream proteins that determine cancer cell phenotypes. Third is the biology and clinical studies that uncovered the roles of these molecules in cancer development (Macaluso, Paggi, & Giordano, 2003). Finally, the latest breakthroughs are the development of sensitive and specific techniques to detect and target these

molecules for the diagnosis and specific treatments of cancers (Fischer et al., 2003; Ross et al., 2004; Sidransky, 2002).

Professor Peter C Nowell is the beginning of this story. Nowadays, it is widely accepted that the principles of oncogenesis include multiple somatic mutations, clonal origin, genetic instability, and multistep processes of selective clonal expansion resulting from mutations in oncogenes and/or tumor suppressor genes (Cahill et al., 1999; Knudson, 1995; Lengauer et al., 1998; Liu & Weissman, 1992; Nowell, 2002; Varmus, 1984). In 1960, he and Professor Hungerford discovered a minute chromosome in human chronic granulocytic leukemia patients (Nowell, 1962). This chromosome was later specified as Philadelphia chromosome. This discovery is the first demonstration of a consistent genetic abnormality in cancer (Nowell, 1962). More importantly, his studies led to the conclusion that single somatic mutation could subsequently lead to other genetic changes. He called this a clonal evolution of tumors model (Cole & Nowell, 1965; Nowell, 1976). This concept is held today as the guiding framework of current understanding of tumor development and forms the basis of the hypotheses regarding cancer stem cells.

Selective clonal expansion as a consequence of mutations in oncogenes and tumor suppressor genes may be the most crucial law of molecular biology of cancer that led to the development of specific diagnostic tools and targeted therapies. Surprisingly, the discovery of both sets of genes was serendipitous. In 1976, the Nobel Laureates Professors Harold Vamus and J. Michael Bishop discovered a chicken SRC oncogene from a copy that recombined with a retroviral genome which was originally thought of as a viral oncogene, but was later proved to be the first identified oncogene (Newmark, 1989). In contrast, tumor suppressor gene was discovered by hypothetical approaches. In 1971, Professor Alfred Knudson proposed that two genetic events were sufficient to lead to a clinically manifested retinoblastoma (Knudson, 1971). These two events hit the two alleles of a single gene. Consequently a gene, normally inhibiting cell growth, was inactivated and tumor predisposition occurred (Devilee, Cleton-Jansen, & Cornelisse, 2001; Gilbert, 1983; Klein, 1987). Six years later W.K. Cavenee and colleagues (Cavenee et al., 1983) showed that, in retinoblastoma, the second hit is usually a chromosomal mutation, such as a deletion, mitotic recombination or nondisjunctional chromosome loss. These two hits lead to LOH over large regions of the chromosome. The “two hits” concept and LOH has been a

core basis of the genetic mapping approach used in the identification of most, if not all, tumor suppressor genes (Yanatatsaneejit et al., 2007; Yokota, Sugimura, & Terada, 1991).

Herceptin and Gleevec are the first therapeutics developed to target oncoproteins. Both are prototypic targeted therapeutic agents in cancer and are currently in use in clinical practice (Ross et al., 2004). While the success of Gleevec was contributed by scientists from several groups, the Prince Mahidol Award Laureates Professor Axel Ullrich has made most of the major contributions for the development of Herceptin. In 1985, Professor Ullrich cloned HER2/c-erB2. In collaboration with Professor Dennis Slamon, they discovered that HER2 is amplified and overexpressed in 25% of all breast cancers (Slamon, Clark, Wong, Levin, Ullrich, & McGuire, 1987). They also demonstrated the correlation of relapse and survival rates with the amplification of the HER2/neu oncogene (Slamon et al., 1987). Professor Ullrich's laboratory then developed several monoclonal antibodies against HER2, one of which, MAb 4D5, was subsequently humanized and developed into Herceptin (Trastuzumab) (Hudziak, Lewis, Winget, Fendly, Shepard, & Ullrich, 1989). Herceptin has had an impact in the treatment of HER2-positive metastatic breast cancer. Recent clinical trials have found that Herceptin reduces the risk of relapse in breast cancer patients (Romond et al., 2005).

Conclusion

History of molecular genetics in cancer concludes that it is important to realize the important of basic research. Without it, there will be no breakthrough in medicine. For example, there would be no Imatinib or Trastuzumab, if there had been no key discoveries such as Philadelphia chromosome and BCR-ABL, a chimeric protein product from the chromosome or HER2/c-erB2, respectively. We are in wrong direction that we, Thai biomedical scientists and granting agencies, are limiting our scopes of researches within immediate applications. I wholeheartedly believe that it is possible for Thai, even under limited resources, to contribute and write history of medicine in the future.

New Discoveries in Chulalongkorn University

In 1994, we started molecular genetics research at Chulalongkorn University to explore cancer biology. Since then, our group has reported several scientific articles, and these articles have received significant number of citations. More importantly, our group

discovered several unique molecular genetic events related to human diseases, and these discoveries have contributed to the advancement of research, public health and medical service. Our research experience may be unique. Molecular and cellular biology research usually requires advanced technologies and large budgets. Research in Thailand usually received relatively limited resources. Nonetheless, our experiments were performed almost exclusively in Thailand by Thai scientists applying basic technologies. This article reports our work and reveals how we could succeed.

First Approach

Our first grant from "The Thailand Research Fund" was to describe frequencies of LOH in nasopharyngeal carcinoma (NPC) chromosomes (Mutirangura et al., 1997). This research provided information for locating important tumor suppressor genes preventing NPC development. We were the first to report genome wide mutations in this cancer. We subsequently reported a total of four follow-up original articles using the same approach (Mutirangura et al., 1999; Mutirangura et al., 1998a; Mutirangura et al., 1996b; Tanunyutthawongese et al., 1996). One of the factors that contributed to the success of this study with a minimum of technical obstacles was that we used the same molecular genetic techniques as those used in my Ph.D. thesis, but for a different research objective. While I was a Ph.D. student at Baylor College of Medicine, USA, in addition to participating in the human genome project, I discovered the mechanism of uniparental disomy (UPD) of chromosome 15 (Mutirangura et al., 1993). Both LOH and UPD studies were based on microsatellite analysis, which is the same DNA fingerprinting method that is currently used for personal identification in forensics. Under normal circumstances, getting started is difficult for a young scientist. My experience suggested that one should start his or her own research by applying the technology that they know best. Moreover, in science, applying a technology from one field to another usually results in a productive outcome.

Discovery by Hypothesis

My first publication in Thailand was to report the frequency of telomerase activity in oral leukoplakia, a type of oral lesion with the potential to develop into oral cancer (Mutirangura et al., 1996a). When cells express telomerase, the cells possess limitless proliferation potential. Telomerase activity was also commonly found in cancer cells, but not

in normal tissue (Kim et al., 1994). Moreover, cancer develops from normal cells via a multistep carcinogenesis process (Vogelstein & Kinzler, 1993). This means that gradual histological changes from normal tissue to premalignant and then malignant tissues can be observed. Because oral leukoplakia is premalignant tissue of oral cancer, it was reasonable to hypothesize that these lesions possessed telomerase activity. This is an example that demonstrates how to have a new discovery by prediction. When there are connections between parameters, a correlation between the shared parameters can be hypothesized. For the telomerase study, not only telomerase and cancer are connected but premalignant lesions and cancer also are. Therefore, an association between telomerase activity and premalignant lesions was hypothesized.

We have used this approach to discover several new molecular genetic characteristics in human diseases. The most important example is the discovery of Epstein Bar virus (EBV) DNA in serum of NPC patients (Mutirangura et al., 1998b). EBV infection of the nasopharyngeal epithelium is one of the prerequisites of NPC (Niedobitek, 2000). Therefore, most NPC cells possess EBV DNA (Niedobitek, 2000). Interestingly, there were several reports that demonstrated a significant amount of cancer DNA in patients' sera or plasma, known as circulating free cell DNA (Chen et al., 1996; Nawroz, Koch, Anker, Stroun, & Sidransky, 1996). Our EBV DNA discovery led to several subsequent evaluations of several groups, for example in Hong Kong and Taiwan, between the quantity of plasma EBV DNA and patients' clinical conditions (Lin et al., 2004; Lo et al., 2000; Lo et al., 1999). Interestingly, plasma EBV DNA disappeared after irradiation, particularly among NPC patients with complete remission. The EBV DNA reappears if the cancer relapses, or persists because of incomplete response to treatment. Therefore, the quantity of plasma EBV DNA has been a useful tool for monitoring NPC patient treatment outcomes (Lin et al., 2004; Lo et al., 2000; Lo et al., 1999).

Discovery from Unexpected Observations

Many scientific discoveries were serendipitous. A classic example of such serendipity was the discovery by Dr. Alexander Fleming of a source of an antibiotic called "penicillin" in 1928 (Bentley, 2005). The discovery was accidental, since the penicillin came from fungi that contaminated his experiment while culturing bacteria. He observed a zone around the fungus where the bacteria could not grow. Subsequently, the fungi were

identified as being from the *Penicillium* genus. The fungi subsequently became the source of penicillin and his unexpected experimental result revolutionized medicine. In contrast to the premise that serendipitous discovery is rare, I believe that many such discoveries have been ignored. Moreover, it may be possible to increase the likelihood of such discoveries.

Recently, we reported the methylation status of endogenous DNA double strand breaks (EDSBs) (Pornthanakasem et al., 2008). We found that EDSBs contain higher levels of methylation than the cellular genome. The differences in methylation levels between EDSBs and the rest of the genome suggest that EDSBs are differentially processed by breakage, end-modification, or repair, depending on the DNA methylation status. If these pathways have different precisions, methylated and unmethylated DNAs should have unequal rates of spontaneous mutation. This study may help explain how cancer genomes, which have genome-wide decreases in DNA methylation levels, mutate faster than normal cells. The mechanism will be important information for further research in cancer prevention. We hypothesized that methylated EDSB processing, such as repair, may be slower and more precise than unmethylated EDSB. I called this hypothesis as “slow but sure” (Mutirangura, 2008). Consequently, hypomethylated genomes are more unstable. Our study is the first report of EDSBs, and there is no current technique capable of identifying such rare sequences. Interestingly, at first, our group did not aim to identify generalized EDSBs, but we were exploring the existence of lymphoid-derived, locus-specific EDSBs, particularly from V(D)J recombination, in NPC. Nonetheless, our first experiment detected EDSBs from all of our negative controls, DNA from normal non-lymphocytic cells. Under normal circumstances, this experimental result would have been thrown away. However, at the time, the potential correlation between EDSB-DNA methylation and global hypomethylation-genomic instability occurred to me.

In conclusion, we believe serendipitous discoveries can happen when scientists observe new events or when use new methods of observation. For example, previous studies observed methylation of LINE-1 interspersed repetitive sequences by Southern blotting and hybridization and found complete methylation of the sequence in normal cells. When we used novel PCR methods, COBRALINE-1 or CU-L1, we found that methylation patterns of the sequences can be varied (Chalitchagorn et al., 2004; Phokaew, Kowudtitham, Subbalekha, Shuangshoti, & Mutirangura, 2008). Moreover, scientists should learn to observe the unexpected and must not ignore such results. Finally, scientists must

be able to connect their findings with potential causes of the unexpected results and understand the importance of the observations. These connections provide the starting hypotheses for subsequent studies.

Conclusion

We reported several unprecedented molecular genetic events including the mechanism of UPD 15 (Mutirangura et al., 1993), telomerase activity in premalignant lesions (Mutirangura et al., 1996a), EBV DNA in NPC patients' sera (Mutirangura et al., 1998b), human papilloma viral DNA in cervical cancerpatients' plasma (Pornthanakasem, Shotelersuk, Termrungruanglert, Voravud, Niruthisard, & Mutirangura, 2001), new NPC susceptibility genes, polymeric immunoglobulin receptor (*PIGR*) (Hirunsatit et al., 2003) and *HLA-E* (Hirankarn, Kimkong, & Mutirangura, 2004), *SHP-1* methylation in normal epithelium and demethylation in psoriasis (Ruchusatsawat, Wongpiyabovorn, Shuangshoti, Hirankarn, & Mutirangura, 2006), promoter methylation of several genes in NPC (Yanatatsaneejit et al., 2008), cervical cancer (Kitkumthorn et al., 2006) and *TTC12* methylation in leukemia (Wattanawaraporn, Singhsilarak, Nuchprayoon, & Mutirangura, 2007), genome wide methylation patterns (Chalitchagorn et al., 2004; Phokaew et al., 2008) and EDSB methylation status (Pornthanakasem et al., 2008). Some discoveries occurred through standard hypothesis testing techniques. UPD, *SHP-1* and *TTC12* methylation, genome wide methylation patterns and EDSB methylation status were serendipitous discoveries (Chalitchagorn et al., 2004; Mutirangura et al., 1996a; Phokaew et al., 2008; Pornthanakasem et al., 2008; Ruchusatsawat et al., 2006; Wattanawaraporn et al., 2007).

Human and molecular genetic research begins with basic research to understand the biology of DNA. This understanding is important to develop novel and better applications in medicine and public health. Therefore, basic research (in this case, molecular genetics) is important, not only for mankind; it will also build the foundation for improving the socioeconomic status of each nation, both directly and indirectly. Basic research is competitive and usually requires large budgets and excellent resources or dependable international collaborations. Nonetheless, I believe the most important tools for success in science are intellect and wisdom. We have followed his majesty the king's address on the topic of "Sufficiency Economy" and believe in this philosophy wholeheartedly (http://www.md.chula.ac.th/biomed/news%20file/เศรษฐกิจพอเพียง.pdf).

Notes: “History of Molecular Genetics in Cancer” was modified from my report in 2007 as a SAC member of Prince Mahidol Award. “New Discoveries in Chulalongkorn University” was modified from “the achievements of Professor Dr. Apiwat Mutirangura” in “2008 Outstanding Scientist Awards” book.

Acknowledgement: All researches in Thailand were supported by the Thailand Research Fund (TRF), National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand and Chulalongkorn University.

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