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From cell differentiation to tumorigenesis

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At the age of 13, I arrived in America with my family from Taiwan. At that time my command of English was limited to the alphabet and schools did not offer “English as a second language” programs. The majority of my teachers allowed me extra time during quizzes and examinations for the reason that I had to look up words to understand the questions being asked. My initial years of schooling were very difficult with the exception of mathematics. I was considered a mathematical “genius” for the fact that one is not required to have a vast comprehension of English to perform calculations. My brother and I learned our intonations from various television shows and, in doing so; we practically memorized all the programs from the 60’s.

I became involved in the sciences towards the start of my junior year in high school. My best friend’s mother was a secretary for Dr. Frank Ruddle at Yale University and asked for assistance to file away reprints. Being that this was my first paying job, I worked diligently to get the work done as fast and proficiently as possible. Dr. Ruddle noticed my assiduousness and asked me to remain in his laboratory to gain knowledge of scientific research. I was thrilled; for a junior in high school that was very “cool”. I remained working for Dr. Ruddle part time during the school years and full time during the summers. During that period, somatic cell hybrids were the primary method in mapping specific genes to chromosomes. I learned basic techniques in gel electrophoresis, sterile tissue culture, the handling of rodents, and the preparation, and staining, of chromosome karyotypes. I worked for Dr. Ruddle for the remainder of my years at high school and college.

Upon my graduation from college with a B.S. in mathematics, I was unsure of my future. I remained in the Ruddle lab for an additional two years. During my time there, I befriended many individuals, many of whom are well known scientists today. The majority of my colleagues suggested that I attain a graduate degree in the field of biological sciences. I ultimately decided to attend Albert Einstein College of Medicine to pursuit a Ph.D. under the guidance of my mentor, Dr. Frank Lilly. Much of the valuable techniques, as well as an acquired intellectual capacity in the field of research from being in the Ruddle lab, facilitated my short but successful graduate studies. While there, my graduate research was focused on the development of spontaneous lymphoma in the progeny of two inbred strains of mice with no known predisposition to lymphoma. We had demonstrated that the onset of spontaneous T-cell lymphoma in the progeny was a result of genetic complementation. I learned mouse genetics from Frank Lilly and, to this day, I remain performing my own animal studies; a practice instilled in me from Frank Lilly.

Subsequent to attaining my Ph.D. in genetics I carried out my postdoctoral training at Columbia University where I began work on cell transformation and DNA

tumor virus, SV40, under the guidance of Dr. Robert Pollack. During my second year of postdoctoral training, Dr. Pollack became Dean of Columbia College. He requested that, in his absence, I remain to manage his laboratory. My responsibilities included, but were not limited to, directing the research activities of seven graduate students in his laboratory. I am proud to say that all of them completed their research requirements, attained their Ph.D.s, and obtained excellent postdoctoral positions. Currently, all of them continue to be productive, successful scientists in various academic institutions and pharmaceutical companies.

During my tenure at Columbia University I worked closely with Dr. Pollack. Together we published several papers and secured a number of grant supports. We were also able to recruit three additional graduate students as well as one postdoctoral fellow. After the conclusion of nine years, Dr. Pollack returned to the laboratory and I began my search for a permanent, independent position.

I was recruited as an assistant professor by Dr. Allan Conney, the chairman of the Department of Chemical Biology, School of Pharmacy, Rutgers University. I have been a faculty member at the School since 1992 during which I ascended through the ranks from assistant professor to associate professor and now to full professor.

While at Columbia University, I became interested in the regulation of cell differentiation. My colleagues and I adopted an adipocyte differentiation system developed by Dr. H. Green and colleagues as our model. Green et al., had previously demonstrated that from an extremely confluent tissue culture plate of Swiss 3T3 cells one can isolate stable clones that either continue to remain as undifferentiated fibroblasts or acquire the capacity to differentiate into adipocytes. These adipocytes were then shown to exhibit the morphological and biochemical characteristic of full-fledged fat cells.

We initiated a series of rather naïve experiments in which we took the genomic DNA from differentiated fat cells and transfected it into undifferentiated sister fibroblastic cells. To much of our surprise the experiment actually worked; we were able to commit fibroblastic cells to undergo adipocyte differentiation via DNA transfer. Subsequently, we were able to show similar results with genomic DNA from human fat. Through utilizing molecular cloning techniques and functional assays we identified two small fragments of genomic DNA each with the ability to induce adipocyte differentiation when introduced into a variety of fibroblasts. Our attempt to determine if proteins were ultimately translated from these small genomic DNA clones was not successful; we were only able to identify a “RNA- like” specie in one of the clones.

When I began my position as assistant professor at Rutgers University I took this project with me. I expanded the studies in whole animals by making transgenics with the cloned DNA with the intention to look for the generation of “obese” mice. One of the clones never gave rise to subsequent transgenic progeny and the other one yielded a total of five independent founders. None of them became fat but one founder showed pigmentation on the ears by 7-8 month of age. As this founder aged, the entire ear became pigmented and thick, suggesting hyperproliferation by pigment cells (melanocytes). Additional pigmented foci could be detected on the legs and under the fur. Biopsies were taken and submitted for histological evaluation. The pigmented foci were identified to be melanoma. This finding was very exciting and I switched the focus of my research to melanoma. With the help from Bill Pavan, Jeff Trent and members of

their labs, we determined a piece of host DNA (about 70kb) was deleted by the integration of the transgene. These studies were performed before the completion of sequencing of human/mouse genome, therefore, we had to rely on chromosome walking and DNA sequencing of BAC (bacterial artificial chromosome) clones containing mouse genomic library to identify the subsequent neighbor clone. DNA sequences from these BAC clones permitted us to search the database for candidate genes. We identified several BAC clone comprises about 1 megabase surrounding the host/transgene region. We identified and cloned three genes within that region. Using each of the candidate genes as a probe, we showed one of them to be differentially expressed between tumor and normal samples. The gene was metabotropic glutamate receptor 1 (Grm1). In order to distinguish between the causes and consequences of aberrant Grm1 expression in tumor samples, a second transgenic line was made with cDNA of Grm1 under the regulation of a melanocyte-specific promoter, Dct (dopachrome tautomerase). This new transgenic line displayed a melanoma susceptibility phenotype nearly identical to the original one. These results demonstrated ectopic expression of an otherwise normal protein in an anomalous cell type is sufficient to induce tumor development *in vivo*. We then extended our studies to the human system. We examined human melanoma biopsies and cell lines, and demonstrated ectopic expression of GRM1 in about 40% of these samples, suggesting that GRM1 may play a role in the onset of some human melanomas.

Grm1 belongs to a family of heterotrimeric G-protein-coupled-receptors (GPCRs), and is predominantly associated with excitatory synaptic neurotransmission in the mammalian central nervous system. The cascading signals activated by the ligand, glutamate, are believed to be equally important in regulating the growth and differentiation of a wide variety of both neural and non-neural cells. Others have suggested possible involvement of GPCRs in cellular transformation. Based on previous studies and results from our laboratory, our working hypothesis is that misregulating a normal neuronal receptor protein, GRM1, to allow its expression in an unnatural cellular environment (melanocytes), triggers pathways that transcribe genes involved in cell growth, cell transformation and ultimately leads to tumor formation.

Activation of various signaling pathways, including the Ras/Raf/MEK/ERK module of the MAP kinase cascade, have been implicated as important steps in melanoma development/progression; however, the upstream signaling molecules (such as receptors), and downstream targets (such as transcription factors) remain largely unknown. Our novel model system supplies one of the upstream signals: Grm1.

Our aims and goals for the next five years are to concentrate on human melanoma cells. Our proposal will focus on experiments that begin to unravel complex networks that result in aberrant expression of genes in human melanoma cells. Specifically, we would like to ascertain if GRM1 expression is required for the maintenance of transformed phenotypes and to test GRM1 for its potential as a therapeutic target in the treatment of melanoma. Ultimately we will be able to begin to examine the mechanisms that bring about the deregulation of GRM1 expression in human melanomas.

My involvement in the pigment cell research field has been intense but brief. If not for the generous encouragement, advice, and valuable tools provided by so many investigators in this field, I feel that my research would not have advanced to where is

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