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Learning to SKI in the lab

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I am convinced that chance has played an essential role in my life as a scientist. What I am trying to say is not that I have been "lucky" in the experiment of the week or the year, but how serendipity has influenced my research throughout the years. Let me start by (almost) the beginning. I received a Ph.D. degree in Biochemistry from the University of Buenos Aires and postdoctoral training in the lab of Arthur B. Pardee at the Dana Farber Cancer Institute, Harvard Medical School. Art permeated, and still does, an exultant love for science that was and is totally contagious. In his lab I learned the basic elements of the mammalian cell cycle. In fact, those were pioneering times for all of us since beside mitosis and DNA synthesis, little else was known regarding events taking place between contiguous cell cycles. Art's ideas were very much ahead of everybody else's. Using protein and RNA synthesis inhibitors, and some math, he correctly predicted the existence of the Restriction (R) Point in the cell cycle (1). The R point marks a position in the late G1 phase at which the normal cycling cells decide whether to enter the S (DNA synthesis) phase under favorable growth and nutrient supply, or to withdraw from the cell cycle and enter a reversible state of quiescence. Less than four months after arriving at his lab, I already had gathered enough data for a manuscript that described the unrestricted growth of cancer cells by loss or the R point (2); I was at the right place at the right time.

Another significant event was the arrival of Judith Campisi to Art's lab. Judy and I became almost instant friends; she and I worked in many lengthy experiments that current students and postdocs would almost immediately shy away. Those were the times in which the analysis of cell cycle transit required the use of both thymidine incorporation every hour for over 40 hrs, and fluorescent cell sorter equipment which was so rudimentary that required fixing for almost every experiment (no Cores were available at that time!).

After leaving Art's lab and moving back to Argentina, I started a research program in breast cancer with a focus on stem cells, a weird topic at that time. Of course, there were no stem cell markers (even now there are still a few of them), but chance also intervened. By using the relatively simple physical method of gradient separations we were able to demonstrate the existence of breast cancer cell subpopulations (3). Even more important, we showed that one subpopulation was able to regenerate the properties of the entire tumor cell population (4). Soon after, I also became interested in melanomas when we discovered that freshly isolated human melanoma cells were constituted by subpopulations with different growth capacities and expression of melanoma antigens (5). Needless to say, our ideas were received by skepticism; at that time, almost nobody believed in the existence of tumor stem cells. So, we had to battle many frustrations and negative reviews for quite a bit of time.

Sadly, Argentina was going through a tumultuous period and the University of Buenos Aires was no exception. I decided to continue my melanoma studies in the States. I joined the Department of Dermatology at the University of Cincinnati because it was known for running a strong program in the basic biology of melanocytes and nevus cells. I am indeed grateful to James Nordlund, at that time the Department Chair, and to my friends and colleagues Zalfa Abdel-Malek and Ray Boissy. With their nurturing, I learned to appreciate the complexities of melanocytes biology; these cells continue to amaze me by their colors, morphology and most importantly, by their potential to develop into a devastating disease.

At UC, I developed a better method for culturing adult melanocytes and melanocytic nevi (6). I quickly found visually appealing changes between proliferating melanocytes and those that have ceased to proliferate and were irreversibly out of the cell cycle (cellular senescence) (7).

My lab was the first to show that upon exposure to growth factors, the senescent melanocytes were unable to activate the mitogen activated (MAPK), extracellular-signal regulated kinase 2 (ERK2) (7). The importance of such findings became evident when years later it was discovered that a mutation in the upstream kinase BRAF, a prevalent mutation in over 70% of human melanomas, generates a constitutively active MAPK signaling pathway (8). For those who are not senescence aficionados, it is important to mention that senescence, either by exhaustion of the replicative lifespan or by oncogene activation, was considered by some as an artifact resulting from culturing the cells in plastic. However, it is now evident that senescence functions *in vivo* as a potent tumor suppressor mechanism (9). I am grateful to Judy Campisi, (a giant in the field of cellular aging), for the many hours she spent discussing melanocyte senescence with me.

My tenure at UC was significant in more than one way. Chance played again a critical role when I met Ed Stavnezer, then a faculty member at the Department of Genetics. Ed had previously discovered the oncogene *c-ski* (named after the Sloan-Kettering Institute), as the transforming gene, *v-ski*, of the defective SKV avian carcinoma viruses. At this point, it is important to clarify the apparently confusing nomeclature mentioned herein; SKI indicates the human protein, whereas Ski is the mouse and c-Ski the chicken proteins respectively.

Knowing my interest in melanoma, Ed and his wife Clemencia Colmenares showed me a stunning experiment. They had infected quail skin with the avian *ski* oncogene and found a surprising proliferation of "black cells". Obviously, these cells were melanocytes; so, I rushed to test, with the help of Ed's antibody, whether human melanomas were SKI-positive. The western blot results were absolutely clear, human melanoma cell lines had large amounts of SKI (over 20-30 times) compared to normal proliferating or senescent melanocytes. Unfortunately, at that time nothing was known on how SKI could regulates proliferation. This, and the fact that I was moving to Baylor College of Medicine, postponed any studies on SKI for some time. I did not forget SKI, though I had a hard time convincing members of my new lab that it was a subject worth pursuing. My insistence paid off, we found using the yeast two-hybrid system and our own melanoma library, that SKI interacts with Smad2 and Smad3, two critical members in the TGF-β signaling pathway, transforming them from activators to repressors of transcription (10).

Soon after, we showed that SKI, which increases *in vivo* in a melanoma-progression manner, also displays alterations in its cellular localization (cytoplasmic SKI also represses Smads by retaining them in the cytoplasm). Significantly, SKI is also a potent activator of β-catenin in melanomas (Reviewed in (11;12). New, unpublished results confirmed that SKI is critical for melanoma proliferation in an animal model.

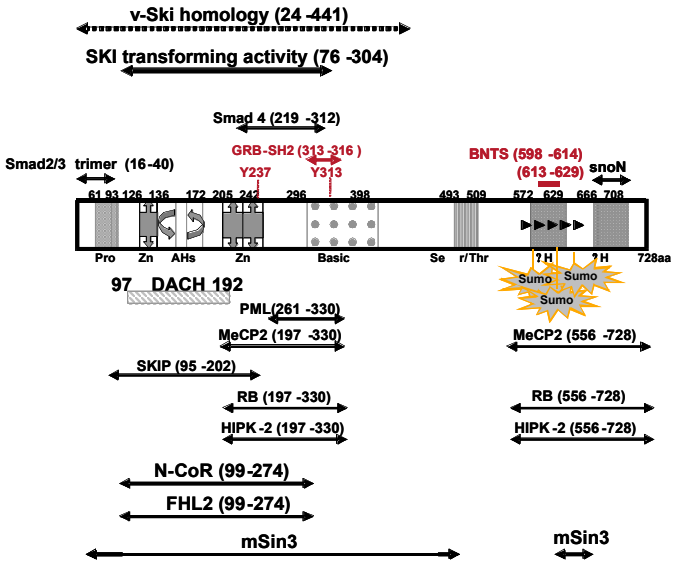

Figure 1: The human SKI protein.
Cartoon depicting motifs and domains required for different protein-protein association. Pro: a proline rich domain; Zn: a leucine zipper-like domain; AHs: helix-loop-helix motifs; Basic: a region of basic amino acids; αH: a unique tandem repeats of alpha helical domains which is involved in the dimerization of the SKI family through coiled-coil interactions. Arrowheads indicate three tandem repeats of 25 amino acids located at residues 572-645. SKI-HD: SKI homodimerization domain. SKI domains required for association with multiple proteins are indicated by a double-arrow line. BNTS indicates a bi-partite nuclear localization signal. Y237 and Y313 are putative phosphorylated amino acids. Of three putative SUMOylation sites localized within the tandem repeats, we found that K573 (Fig. 10) is required for SKI protein stability (unpublished results).

So, we will SKI in the lab well into the future!

I am immensely grateful to James Smith for offering me to continue our senescent studies at the Huffington Center on Aging, Baylor College of Medicine. There, I found outstanding colleagues and a most creative environment for querying the role of chromatin in cell and tissue aging. One of such studies, described in a manuscript under revision, highlights the role of chromatin remodeling proteins in the senescence of melanocytes in culture and melanocytic nevi *in vivo*.

These studies have been tremendously gratifying; they promise to be instructive in the search for clues to the intricate relationship between aging and cancer of the pigment cells (13).

Acknowlegments.
The aforementioned studies would not have been possible without the work from outstanding past and present young scientists and graduate students in my lab. Quoting Renato Baserga (14), "in teaching them, my knowledge grew". I am also grateful to the NCI, NIA and Ellison Medical Foundation for their continuous support.

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