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My Journey to the Land of Melanocortins
How It Started and Where It Is Leading

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My journey to pigment cell research started when I arrived in Tucson, Arizona, a foreign student seeking enrollment in a graduate program in Biology. Fate led me to Mac Hadley, then Professor of General Biology, specialist in Endocrinology, and international figure in comparative pigment cell biology. In the Hadley laboratory was my first exposure to integumental pigmentation, its regulation and physiological significance. It was also my first encounter with the incredible melanocortins. I was fascinated by the immediate skin darkening and lightening of frogs and lizards, and by learning that _-MSH, the physiological regulator of pigmentation in these organisms, also regulates mammalian pigmentation, as was best demonstrated at that time in mouse coat color and mouse melanoma cell lines. Living in Tucson and doing part of my Ph.D. research in the Cancer Center in the laboratory of Frank Meyskens made me aware of melanoma as a challenging form of cancer, caused in most cases by excessive sun exposure. My Ph.D. research focused on investigating the regulation of mouse melanoma cells by _-MSH and various synthetic analogs of _-MSH, including the best known analog [Nle^4, $D\text{-}Phe^7$]- _-MSH (NDP-MSH). My research experience as a graduate student taught the value of collaborations, as best exemplified by the long term collaboration between Victor Hruby, a worldwide authority in Peptide Chemistry, and Mac Hadley, a superb biologist.

The second phase of the journey began in Cincinnati. The ticket to Cincinnati came through Joseph Bagnara, who directed to a postdoctoral position in the Department of Dermatology, then chaired by James Nordlund at the University of Cincinnati. What attracted me mostly to this position was the chance to investigate the regulation of human pigmentation using primary human melanocyte cultures. At that time, only a handful of laboratories worldwide were involved in culturing melanocytes from human skin. I was ready for this challenge, and my research focused during the first few years on identifying the melanogenic factors that account for differences in skin pigmentation, using melanocytes cultured from donors with different pigmentary phenotypes. Subsequently, my research focused on investigating the hypothesis introduced by James Nordlund that a symbiotic relationship exists among different epidermal cells, namely keratinocytes, Langerhans cells and melanocytes, what Jim liked to refer to as **K-L-M**. The idea of a paracrine/autocrine network in the skin was quite novel and even controversial in those days. However, shortly after our first publications on the production of the primary immune-inflammatory cytokines interleukin-1α and β by human melanocytes, and the effects of these two cytokines, as well as interleukin-6 and tumor necrosis factor _ on these cells (1, 2), this idea was adopted and further investigated by other laboratories. Now it is an undisputed concept.

As a junior faculty member, I had to start paving my own independent path in research. I kept communicating with Mac Hadley who at that time was involved in conducting the first clinical trials to test the efficacy of NDP-MSH in inducing "sunless tanning". Mac kept urging me to test melanocortins on cultured human melanocytes. He was a firm believer that human melanocytes, the same as other mammalian melanocytes and vertebrate melanophores, are regulated by _-MSH. I realized that the obstacle to generating a response to _-MSH by cultured human melanocytes was the culture conditions used at that time. By modifying these conditions, and excluding cholera toxin and isobutyl methylxanthine (both known to mimic _-MSH as they increase cAMP formation) from the culture medium, we could readily obtain a response not only to _-MSH, but also to ACTH, much to our surprise at that time. I presented our preliminary results on melanocortins and their effects on cultured human melanocytes for the first time at the 15th IPCC in London, 1993. Anthony Thody's group had similar findings that were presented during the same meeting. The first publications on the effects of melanocortins on human melanocytes *in vitro* came from Thody's laboratory in 1994 and mine in 1995 (3, 4,5). Research on melanocortins became a major subject of investigation in my laboratory, and opened the doors to collaborations with prominent figures in the field of pigment cell research, such as Vincent Hearing, Greg Barsh and Shosuke Ito. The foundations for this area of research were established in collaboration with visiting scientists from POLA Chemical Company in Japan. The accomplishments of this collaboration proved one more time the significance of the alliance between academia and industry, which can enhance scientific progress. The major accomplishments of these collaborations can be summarized as follows:

1. α-MSH and ACTH have equal potency in stimulating human melanocyte proliferation and melanogenesis, and are more potent than β-MSH or γ-MSH (6).
2. The effects of α-MSH and ACTH on human melanocytes are mediated by binding and activating the melanocortins 1 receptor (MC1R) (6).
3. Agouti signaling protein is the antagonist of the MC1R that is expressed on human melanocytes (7).
4. Expression of the *MC1R* mRNA is up regulated by α-MSH, ACTH, endothelin-1, and basic FGF, and down regulated by agouti signaling protein (6, 8, 9).

Melanocortins have taken center stage as important regulators of pigmentation as well as many other physiological and metabolic processes in humans. This started in the early 1990's with the cloning of the MCR genes, 5 genes (MC1R-MC5R) that code for 5 different receptors that differ in their tissue distribution and in some cases in their affinity to the different melanocortins. Secondly, came the finding from several laboratories that melanocortins do not always function as endocrine factors, but are made locally in various peripheral tissues, including the skin, where they are synthesized by epidermal keratinocytes and melanocytes. Thirdly, came the demonstration that normal human melanocytes express functional melanocortin 1 receptors.

In my first NIH R01 grant in 1994, I hypothesized that melanocortins prevent melanoma formation by reducing the burden of UV-induced DNA damage, and enhancing DNA repair. The state of knowledge at that time was that _-MSH increases eumelanin synthesis, thus contributes to the photoprotection of the skin, and that the melanogenic response of human melanocytes to UV is mediated to a large extent by melanocortins (10). Although this still holds true, however, recently we discovered that melanocortins reduce the damaging effects of UV on

human melanocytes by mechanisms that are independent of melanogenesis. We found that α-MSH enhanced nucleotide excision repair and reduced the generation of hydrogen peroxide by UV-irradiated human melanocytes (11). These effects are expected to reduce genomic instability, and hence the chance of malignant transformation of melanocytes to melanoma.

A conclusion that can be drawn from our studies with melanocortins is that they participate in the response of melanocyte to stress, enabling melanocytes to withstand the noxious effects of UV radiation. The role of melanocortins in the stress response has been previously suggested by Andre Slominski and others. We have found that α-MSH reduces the cytotoxicity of hydrogen peroxide on human melanocytes, suggesting that it reduces oxidative stress. I expect that melanocortins function in alleviating cellular stress not only in melanocytes that express MC1R, but also in other cell types that express other forms of the MC receptors, such as neurons and adipocytes.

The *MC1R* gene is now recognized as a melanoma susceptibility gene. Evidence for the connection between melanocortins, the MC1R and melanoma was provided mainly by population genetic studies that identified certain *MC1R* alleles that are strongly associated with red hair phenotype and increased risk for melanoma. Currently, the *MC1R* gene is known to be highly polymorphic and to contribute tremendously to the diversity of human pigmentation. I have been quite intrigued by these findings, and became interested in elucidating the impact of *MC1R* variants on the function of human melanocytes and their response to UV radiation. Data that we derived from experiments using cultured human melanocytes expressing various alleles of the *MC1R* gene show that the *MC1R* genotypes that result in loss of function of the receptor compromise the ability of melanocytes to repair UV-induced DNA photoproducts and reduce generation of hydrogen peroxide (11). These findings offer an explanation for increased melanoma risk of individuals harboring certain *MC1R* alleles.

My interest in investigating the regulation of human pigmentation stems primarily from its significance in determining the risk for skin cancer, particularly melanoma. Our studies on melanocortins, together with the currently accepted notion that the *MC1R* gene is a melanoma susceptibility gene, led me to propose that melanocortins can be exploited in a melanoma preventative strategy. This resulted in a collaboration with James Knittel and Carrie-Haskell Luevano, who designed and synthesized n-capped tetrapeptide analogs of α-MSH. We have tested the potency of these analogs against that of α-MSH in stimulating tyrosinase activity in human melanocytes. We then chose the two most potent analogs to demonstrate that they mimic α-MSH in limiting the UV-induced DNA damage and function as true MC1R agonists. Owing to their low molecular weight and their lipophilicity, these analogs can potentially be developed into topical agents that prevent the damaging effects of sun exposure, immediately, by enhancing DNA repair and reducing oxidative DNA damage, and latently, by increasing eumelanin synthesis (12). This translational research represents the outcome of thirteen years of basic research on the effects of melanocortins on human melanocytes and the significance of the MC1R in the response of these melanocytes to UV radiation. Futurre success of this translational research project should have an enormous impact on human health by reducing the melanoma epidemic, and well as the incidence of nonmelanoma skin cancer.

Ongoing and future experiments in my laboratory aim at elucidating the molecular targets for melanocortins, particularly those involved in DNA repair, oxidative stress response, and melanogenesis. Further, we are continuing our investigation of the impact of various *MC1R* alleles on the responses of melanocytes to melanocortins and UV. The latter can be best achieved by the collaboration between scientists and physicians. Our partner in this research is Sancy Leachman, the Director of the Hutsman Cancer Center at the University of Utah.

An academic career in scientific research is filled with obstacles created mainly by the dwindling sources of funding. However, the joy and excitement of scientific accomplishments outweigh the heartache and headache. What I cherish mostly in my career is my personal interactions with other scientists with whom I had the fortune to collaborate and befriend. The Pigment Cell Societies have created nurturing grounds for productive collaborations and interactions amongst its members. I owe my success to my collaborators and mentors, and mostly to all the young scientists that I had the pleasure and good fortune to mentor. Two longterm companions in this journey have been Viki Swope, and currently Ana Luisa Kadekaro. I believe in leading by example, and my hope is to succeed in instilling the devotion to science and enthusiasm about research to insure the continuity of my profession and beloved specialty.

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