PASPCR Commentary: Melanocyte dendricity, melanosome transfer, and signaling mechanisms involved in these processes

The road less trodden has many stones…

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July 1, 2006

My interest in melanocyte biology began when I was a fellow in Dermatopathology at Yale University in the late 1980's when Dr. Ruth Halaban was kind enough to let me work on a project examining the expression of basic fibroblast growth factor (bFGF) in human skin in vivo. Of the many things she taught me, one of the most valuable was how to culture human melanocytes, a skill I brought with me to The University of Rochester in 1989. My interest in melanocyte dendricity and melanosome transfer as a research focus were in part opportunistic, and in part due the emergence of new information on cytoskeletal reorganization in response to hormones that were being published at the time by Alan Hall and his group that could be applied to theses questions. While many excellent laboratories were studying the basic biology of pigmentation, melanosome biogenesis, and regulation of growth in melanocytes and melanoma, relatively few groups had a dedicated program investigating two other important aspects of melanocyte biology, dendricity and melanosome transfer. My training as a pathologist made analysis of morphology a skill set that could be applied to these sorts of questions. My initial research efforts in my early years at the University of Rochester were in a somewhat related field examining melanocyte migration and adhesion to matrix proteins, and the inter-relationship between melanocytes and keratinocytes in the developing epidermis. Important discoveries included the documentation of integrin expression by melanocytes and the contribution of individual integrins to melanocyte migration. These early papers established that integrin expression is developmentally regulated in human melanocytes and identified the binding domains used by specific integrins to fibronectin, a complex matrix protein that is abundantly present in the migratory pathway of melanocytes (Scott et al., 1992). Anne Haake and I also established that the position and number of the melanocyte within the developing epidermis is dictated by the state of development of the keratinocytes (Haake and Scott, 1991; Scott and Haake, 1991). The property of the keratinocytes to dictate position and number of melanocytes within the epidermis is conceptually similar to recent work presented at the Society of

Investigative Dermatology meeting in May of this year by Dr. Yoshida (Kao Corporation, Japan) in which melanosome distribution and skin color in artificial skin constructs were determined by the origin of the keratinocytes from either a highly pigmented or a lightly pigmented individual.

A hallmark of melanocytic cells is their ability to form dendrites in response to growth factors and to ultraviolet irradiation. Their remarkable plasticity is both an inducement to study them (they are really quite pretty) yet a challenge as interpretation of shape changes must be made in the context of individual cell variation, culture conditions, and other probably unknowable factors. In the mid to late 1990's I turned my attention to examining the signaling intermediates and factors that regulate human melanocyte dendricity and I paid particular attention to the role of the small GTP-binding proteins Cdc42, Rac and Rho in these processes. These proteins link hormones with characteristic changes in the cytoskeleton in virtually all cells examined, so it seemed likely that they would mediate shape change in human melanocytes in response to hormones and ultraviolet irradiation. Rac activation is associated with lamellipodia formation; Rho activation is associated with stress fibers, and Cdc42 activation is associated with filopodia formation. A potent stimulus of melanocyte dendricity is α-MSH, in which the stimulation of the cAMP/PKA pathway is a primary signaling pathway. We showed that the cAMP-PKA signaling pathway regulates the activity of the small GTP binding proteins Rac and Rho in melanocytes, thus providing an explanation for the ability of this pathway to stimulate melanocyte dendrite formation (Scott, 2002; Scott and Leopardi, 2003; Scott and Cassidy, 1998). Elevation of cAMP stimulates Rac activity, resulting in lamellipodia formation and dendricity, and inhibits Rho, resulting in the dissolution of stress fibers, in melanocytic cells. The introduction of dominant negative Rac mutants blocked melanocyte dendricity in response to α-MSH, whereas microinjection of C3 *botulinum* exoenzyme toxin, an agent that specifically inactivates Rho, induced dendricity in human melanocytes. We conclude that cAMP-mediated dendrite formation in melanocytic cells is mediated through upregulation of Rac activity and down-regulation of Rho activity. The importance of Rac and Rho as a downstream signaling intermediate has also been demonstrated by work performed recently in our laboratory in which we have shown that secretory phospholipase A_2 (see below) mediates melanocyte dendricity through the balance of Rac and Rho activity.

In an effort to determine the mechanisms that underlie dendricity in response to ultraviolet irradiation, one has to assume that paracrine or autocrine factors are at work. In the last several years we have begun to examine the synthesis and effects of prostaglandins and phospholipases and their receptors in melanocyte dendricity. Prostaglandins are potent lipid hormones that activate multiple signaling pathways resulting in regulation of cellular growth, differentiation and apoptosis. Phospholipases are enzymes that release phospholipids and free fatty acids, including arachidonic acid, a precursor of prostaglandins, from target membranes. In the skin, prostaglandins are rapidly released by keratinocytes following ultraviolet radiation and are chronically present in inflammatory skin lesions. We were the first to show that human melanocytes express several receptors for prostaglandins, including the PGE_2 receptors EP_1 and EP_3 and the $PGF_{2\alpha}$ receptor FP (Scott et al., 2005; Scott et al., 2004). We showed that $PGF_{2\alpha}$ through the FP receptor, stimulates melanocyte dendricity as well as the activity and expression of tyrosinase, the rate-limiting step in melanin synthesis. Analysis of FP receptor regulation showed that the FP receptor is regulated by ultraviolet radiation in melanocytes *in vitro* and in human skin *in vivo*. We also showed that ultraviolet irradiation stimulates production of $PGF_{2\alpha}$ by melanocytes, suggesting that $PGF_{2\alpha}$ is an autocrine factor for melanocyte dendricity. These data really represent the tip of the iceberg in terms of prostaglandins and human melanocytes. I hope that others will take up the cause and define in detail the potential synthesis of other prostaglandins and related compounds such as leukotrienes by melanocytes, and their potential regulation by ultraviolet irradiation.

The role of phospholipases in melanocyte biology remain largely unexplored. Secretory phospholipases comprise a large family of Ca^{+2}-dependent enzymes that liberate arachidonic acid as well as lysophospholipids. The predominant secretory phospholipase expressed by keratinocytes is group X $sPLA_2$, which liberates large amounts of arachidonic acid and the lysophospholipid lysophosphatidylcholine (LPC), from membrane preparations. We treated human melanocytes with recombinant $sPLA_2$-X and found that low levels of $sPLA_2$-X stimulate both tyrosinase activity and melanocyte dendricity. We found that the effects of $sPLA_2$-X are mediated predominantly by LPC, not arachidonic acid, and we have demonstrated expression of the phospholipase A2 receptor and two G-protein coupled receptors for LPC (G2A and GPR119) in human melanocytes. Because secretory phospholipases are released during inflammation and are regulated by ultraviolet irradiation, our data suggest an important role for $sPLA_2$-X in

cutaneous pigmentation through the release of LPC. This work has brought to light an interesting function for the zeta isoform of protein kinase C. The PKCζ isoform is of particular interest as a potential mediator of melanocyte dendricity because it has been implicated in cell migration and cytoskeletal reorganization in other cell types, in part through phosphorylation of profilin, and in determination of cell polarity (Izumi et al., 1998; Sanz-Navares et al., 2001; Vemuri and Singh, 2001). Of particular interest are reports of the association of PKCζ with the small GTP-binding proteins Rac and Cdc42. Our recent work has shown that inhibition of PKCζ results in abrogation of the effects of LPC on melanocyte dendricity, indicating a specific function for PKCζ in melanocyte dendricity. Further, LPC activates Rac in human melanocytes, followed by activation of PKCζ. A model in which secretory phospholipase releases LPC from membranes, followed by activation of Rac and PKCζ may explain the mechanism underlying melanocyte dendricity in response to $sPLA_2$-X.

Insight into the signaling intermediates and method of melanosome transfer remains largely a mystery. It is generally assumed that ultraviolet irradiation stimulates transfer, but whether this is due predominantly to the effect of UVR on dendricity or as a separate effect on transfer itself is not known. A major hurdle that has severely limited progress in understanding the molecular basis of melanosome transfer has been the lack of a model system. In 2002 we employed high-resolution digital movies, enhanced through MatLab processing, of human melanocyte-keratinocyte co-cultures, to show that filopodia are conduits for melanosome trafficking(Scott et al., 2002). These experiments were tedious in the extreme, due to the necessity of culturing 2 cell types, keeping them healthy for the duration of the imaging, and technical issues regarding imaging of live cells on an upright microscope. After hours of analysis of these movies my colleague Brian Madden and I came to the conclusion that melanosomes move along filopodia and from filopodia tips they enter keratinocytes (Scott et al., 2002). Filopodia are very thin actin-based structures that project from the cell membrane and are present in virtually all cells in culture. Our movies demonstrated these structures arising from the tips and sides of melanocyte dendrites and we showed that melanosome move along these structures into keratinocytes. Previous to this, knowledge of the mechanism of melanosome transfer had been based almost solely on electron micrographs, which gave only limited information. We also demonstrated that Cdc42, a small GTP binding protein involved in actin assembly, mediates filopodia formation in melanocytes, and hence melanosome transfer. Based on these movies we

propose that melanosome transfer occurs when filopodia tips are engulfed through a "microphagocytosis" modality by keratinocytes. While this model system has the powerful advantage of allowing one to visualize transfer in real time, it is unable to provide quantitative data, which limits is usefulness. However, my general impressions of melanosome transfer to keratinocytes, at least in tissue culture, is that it is a random and haphazard event, filled more often than not with failure than with success. The connections between filopodia and keratinocytes membranes was a times transient, and at other times more "permanent" structures resembling dendrites appear to arise from filopodia that had become securely fastened to the keratinocytes membrane. Work done by and in collaboration with Dr. Miri Seiberg and colleagues at Johnson and Johnson helped to elucidate a potential receptor for phagocytosis of melanosomes contained within filopodia tips. The proteinase activated receptor –2 (PAR-2) is involved in melanosome transfer and skin pigmentation and has been described in a recent commentary by Dr. Seiberg so I won't go into it extensively in this discussion. Suffice to say that we showed that this receptor is upregulated by ultraviolet irradiation in keratinocytes *in vitro* and in skin *in vivo*, and that PAR- 2 dependent phagocytosis in human keratinocytes is Rho-dependent. Together these studies suggest that PAR-2 is a critical receptor involved in keratinocyte uptake of melanosomes. Many questions remain to be answered about the mechanisms and stimuli involved in melanosome transfer, and ingenious solutions must be applied to the problem of establishing a workable and quantitative model for melanosome transfer.

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