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Melanocyte biology: the well-kept secret

At the end of July this year I attended a Gordon Conference on epidermal keratinocytes and felt rather lonely as the only melanocyte biologist. There were these 10+ groups happily (or rather heatedly) arguing over the virtues of keratinocyte stem cell marker A versus B. Every conceivable keratinocyte gene was knocked out in mice, and I started to roll my eyes about the 'interesting' pathologies of artificial mouse diseases. In a coffee break I walked over to Carl Barker, the new SRA for NIAMS, and asked him how many grants were being supported in pigment cells by his institute. We both thought there were less than a dozen. I did not dare to ask about the number of keratinocyte-related grants, and I never checked the NIH databases because I got too sad about the small group of researchers holding on to study this cell type as a career goal. Thanks to the strong intramural group in melanocyte biology, with additional excellent research pockets in Japan and Europe, the overall picture is not too depressing. Still the melanocyte biology field is, in comparison to other cell-associated fields, exceptionally small. Is this good or bad for the few active laboratories in the US? I think it is bad for our collective knowledge.

Melanocytes control the color of our skin. Thus, no other cell product is more looked at by us than the pigment that melanocytes produce. My mentor Wallace Clark gave a fitting description of the importance of skin and skin color in a chapter on pigmented lesions, which I quote in the reference because it is difficult to obtain (1). Each year consumers spend billions of dollars wanting to change the color of their skin from light to dark or dark to light. Despite the strong interests by the average persons in their skin appearance, there is a surprising paucity of research investigations into our understanding of how the melanocytes manage their self-renewal, how they migrate along the basement membrane, arrest and do their apparently only job: produce pigment and pump it.

Human melanocyte biology got off the ground after Magdalena Eisinger published in 1982 her use of TPA as a melanocyte mitogen. In the mid-eighties I learned to isolate melanocytes when visiting the lab of Tom Masiak, then at American Red Cross. However, we had our doubts whether these cells were really normal or whether TPA had transformed them because cells expressed melanoma-associated markers such as ß3 integrin or chondroitin sulfate proteoglycan, which were not found on cells in skin (2). Our relief came, when Istvan Valyi-Nagy came to the lab in the late eighties to co-culture melanocytes with keratinocytes and found that the epithelial cells are the dominant cell over the melanocyte phenotype (3). Direct cell-cell contact was required for control by

undifferentiated keratinocytes over melanocyte growth and expression of cell surface molecules, but the matrix was enough to control cell shape.

This finding should have encouraged us to search for the mechanisms of how the keratinocytes could be so dominant over melanocytes and which pathways were most important to either stimulate or stop growth of the pigmented cells. Grant deadlines and reviewers' demands pushed us in other directions. I had to care for an increasing number of co-workers and there was a never-ending stream of deadlines. I should have taken a sabbatical and tackled this puzzle, but my hands-on skills were rusty by then. The wiring between melanocytes and keratinocytes remains a mystery and an opportunity for future research. Barbara Gilchrest had reported in the early nineties on a glue between melanocytes and keratinocytes, E-cadherin, that appears essential for cell-cell control, but our tools at that time were insufficient to take full advantage of this finding. The power of keratinocytes was clearly demonstrated when Mei-Yu Hsu overexpressed E-cadherin in melanoma cells. Suddenly, a highly metastatic cell with more than 100 chromosomes was tamed and moved at the tune of the normal epithelial cells (4). Could this be a therapeutic strategy? Not quite, because we would need to first upregulate E-cadherin in the melanoma cells followed by sending in the keratinocytes as police. On the other hand, one could design or screen for mimetics of keratinocyte-induced control over E-cadherin overexpressing melanoma cells. Such mimetics could be derived from small molecule libraries and they could guide us to discover the signaling mechanisms.

Disrupting melanocyte-keratinocyte interactions is likely the pre-condition for proliferation, and E-cadherin is readily downregulated by growth factors produced by either keratinocytes of fibroblasts. When Carola Berking overexpressed growth factors in the dermis, we could demonstrate the havoc dermal cells can play on melanocytes (5). Our pathology colleagues had 'difficulties' distinguishing those growth factor-induced lesions from invasive melanomas in patients. Of course we needed to use cocktails of growth factors, but they were easily identified based on extensive growth studies worked out in the labs of Ruth Halaban, Zalfa Abdel-Malek, Dot Bennett and Estela Medrano, and were confirmed in mouse genetic studies in which endothelin, Wnt and stem cell family members were knocked out. From the keratinocyte field we know of the powerful role that the dermal cells have for the epithelial cell in epidermis. The same will be true for the epidermal melanocytes. The growth factor studies demonstrated the powerful role that stromal cells in the dermis have for the epidermal melanocytes, but growth factors cannot induce malignant transformation. Thus, several groups have transformed cells with oncogenes and disrupted the functions of tumor suppressor genes. In the eighties we had used a sledgehammer approach, the SV40 virus, but never liked it because of the severe changes that the virus induces in cells. SV40T antigen remains an often-used tool for transformation, which we still find a poor choice because it has nothing to do with natural transformation of melanocytes in humans. We now have preliminary data that it is enough to disturb two genes to transform melanocytes, but much needs to be learned as to what really happens in skin of patients. Despite all the epidemiological data connecting skin color and sun exposure to melanoma, there continues to be strong suggestions for a UV-independent mechanism for melanoma development, at least in those patients who develop the disease on non-sun exposed skin sites.

For many years, I did not want to work on pigmentation. There was a block, which I explained to myself came from the German recent history of judging people by their color and shape, and I never wanted to participate in anything remotely associated with

changing people's skin color. So far, I have indeed never actively worked on pigmentation genes, but the old excuse has no rational basis. The field has learned a lot about the risk for melanoma development due to point mutations in the MC1R gene. Since I grew up as a carrot head, Rick Sturm, when he did a sabbatical in the lab, volunteered to sequence my MC1R gene. Of course I had two mutations and I guess he still has the data in his notebook because I forgot about them.

Microarray studies on cells from melanocyte co-cultures with keratinocytes had pointed Mizuho Fukunaga to the matricellular protein CCN3, which is not an adhesion ligand itself but is an emergency mediator for melanocytes to maintain contact with the basement membrane (6). There is continuous danger of an 'up-and-out' for the cells, i.e. if melanocytes lose their anchorage to the basement, they have only two choices: undergo apoptosis or transform. When we found a paper describing the downregulation of the laminin receptor alpha 6 beta1 in melanocytes after UV exposure, we realized there must be a second mechanism. It goes like this: Sun exposure or any inflammation induces pro-inflammatory cytokines such as IL-1 in keratinocytes. IL-1ß induces strongly the expression of CCN3 in melanocytes, which then upregulate a collagen IV receptor, DDR1. This receptor tyrosine kinase appears to jump in for the laminin receptor to secure anchorage. This is another example of the intimate crosstalk between epidermal cells.

There are different types of pigmented cells in different organs fulfilling a variety of functions. In skin, they seem to be there for the sole purpose of pigment production and sun protection. Are they all the same? Of all the other cell types one may study from skin such as fibroblasts, keratinocytes, or endothelial cells, we know that there are different flavors for each. Stanford's Howard Chen recently compared the diversity of fibroblasts to that of hematopoietic cells. For each of the above cells there are also stem and progenitor cell populations. Cells in different body sites may have different phenotypes. We still treat epidermal melanocytes as a uniform population, but the picture becomes more diverse as Hong Yu found melanocyte stem cells in human hair follicles (7). We are currently working on a stem cell in the dermis that we can differentiate to melanocytes. Will they replenish the epidermal population in times of need? Can and will they transform to melanoma? Questions we need to address.

Historically, our lab's major focus was on melanoma. In the late seventies and early eighties we had produced monoclonal antibodies, which continue to this day to be outstanding research tools, but in melanoma they failed to be the 'magic bullet' for therapy. We had been disappointed with monoclonals and turned to tumor biology studies. In the late nineties I met a charismatic person, who had metastatic melanoma. Noreen O'Neill personified those patients who have little choice but hope for a miracle. All the therapies the field had come up with did nothing to prolong survival and nobody talked of cures. Together we started the Foundation for Melanoma Research, which today bears her name. When Noreen died of melanoma, her sister Kate took over and has been a strong advocate for melanoma research, whose impact goes way beyond her foundation. When the mutation in the BRAF oncogene was published in 2002, we jumped at the opportunity and developed a therapy program that now occupies 50% of our laboratory efforts. Activated BRAF was a welcome enzyme for industry and today every major drug company that I know of has a BRAF targeting program. We agree that BRAF is a superb target for therapy, but we are learning the hard way that targeting one protein in a pathway is not sufficient to achieve cures, even in animal models. We need to also target other pathways that may function as alternative or escape. How many

inhibitors are needed? It depends on the individual patient and his/her activation profile. We need to establish for each patients' lesions a genetic profile because it will guide us to how the tumor is driven. Then we need to develop an activation profile for the most important pathways because we do not yet know whether and how mutations translate into pathway activation. Will industry lead the way toward personalized therapies? I doubt it because it is a hard way to make a profit. I think that academia will have to jump into the cold water of drug development and perhaps develop partnerships with industry for areas such as medicinal chemistry.

There are many research areas where the melanocyte field can excel because the skin is so easily accessible. For example, two-photon microscopy can trace cells in living skin at the single cell level. We can produce synthetic skin that can take over many functions for normal human skin. Where else but in skin can you study through a chemical biology how cells talk to each other. Melanocytes are an outstanding example for experimental investigations because we can now follow all their activities in health and disease. We need more people in the field to tackle the many pressing questions from different angles. We need multidisciplinary consortia and research networks instead of confining the work to ourselves. We need the talent and enthusiasm of every young researcher and clinician who we can find.

References and see the laboratory website for more details http://www.wistar.org/herlyn

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**Man, having certain advantages over the ameba, uses another derivative of his neuroectoderm, the central nervous system, to correct his native cutaneous decorative flaws. And does he correct the flaws! Down through the relentless march of years from early Pleistocene to the ubiquitous cosmic counters of this very day in Holocenen time, H. sapiens has painted his skin; draped strands and bits of metal over its narrow places and outcroppings; punched holes in it; covered it with metal and stones of all descriptions; made it lighter if it was dark and darker if it was light; cut the hair, let the hair grow, colored the hair, abandoned the hair, and covered the skin with cloth, leather and fur of unending variety and color. Now, take your decorated self back to Noah's Zoo (take your drink and melted brie with you) and stand with some pride between the common peacock and the giant panda. Your conspecifics will now give you notice and you him. Much of your daily time is spent looking at the decorations H. sapiens uses and in formulating your own integumentary modifications. The skin is the way we present ourselves to ourselves and to each other. The skin is the cosmetic organ. Man spends much of his time, creativity, and money in altering the drabness of his integument. The decorative act is a common behavioral thread firmly linking creatures of diverse cultures into a common cosmetic union. Man is a creature of adornment.*

Considering the imperatives of appearance in human relationships, it is not surprising that an alteration in the appearance of the skin may be the single most important feature of cutaneous disease. Many cutaneous diseases have minor symptomatology, while others have none at all. Few are life-threatening and many are self-limited. (There are notable exceptions to this statement.) However, even the self-limited, asymptomatic cutaneous diseases are of great concern to the patient. For example, the symptoms of acne are minor, but the disease can totally change a life. Vitiligo, a completely asymptomatic, progressive mottled depigmentary disorder, commonly converts a normal human being into a recluse. Loss of some unneeded scalp hair has generated an industry of significant size.