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Meeting Report

Melanoma Biology and Progression

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A group of 25 investigators met on November 6–8, 2000 in Bethesda, MD for the purpose of accelerating progress in understanding the biological events in melanoma as they relate to etiology, immune response, and progression. The format of the meeting was the presentation of brief reports that focused on concepts rather than specifics, with extensive discussion periods to identify the issues and barriers hindering progress in the field. This report summarizes the findings of the meeting, highlighting the recent advances in understanding melanoma development and progression and addressing opportunities for better diagnosis, prognosis, and therapy. Several specific recommendations are made to strengthen the field and advance knowledge and progress.

The meeting started with overviews on melanocytes and melanoma. This session was chaired by Meenhard Herlyn (The Wistar Institute, Philadelphia, PA). Dorothy Bennett (St. George's Hospital, London, United Kingdom) summarized the current information on melanocyte development, homeostasis in the adult skin, and the role for the *INK4A* tumor suppressor gene in senescence. Margaret Tucker (National Cancer Institute, Bethesda, MD) presented epidemiological data of genetic and environmental causes of melanoma. The *p16* gene alterations are important in the development of familial melanoma but apparently not for sporadic melanoma. Main risk factors for melanoma are the total number of nevi, number of dysplastic nevi, color of hair, skin, and eyes, and sun exposure during childhood. David Elder (University of Pennsylvania, Philadelphia, PA) outlined the different stages of tumor progression in melanoma, particularly the transition from a biologically early stage of primary melanoma of the radial growth phase, which has no propensity to metastasize, to the advanced primary melanoma of the vertical growth phase, which is associated with increased risk for metastasis. Global gene expression profiling of melanoma, outlined by Jeffrey Trent (National Human Genome Research Institute, Bethesda, MD) promises to help not only melanoma diagnosis but also understanding the biological events during progression.

The session on melanoma models was chaired by Mary Hendrix (University of Iowa, Iowa City, IO). It dealt with both human and mouse models. Human skin grafted to immunodeficient mice, presented by Meenhard Herlyn, provides an orthotopic environment for both melanoma growth and for induction of melanoma by UV irradiation. Organotypic cultures of human skin with an artificial dermis of fibroblasts in collagen and an epidermis of keratinocytes and melanocytes allow the study of gene functions in a three-dimensional tissue environment. Honnavara N. Ananthaswamy (M. D. Anderson Cancer Center, Houston, TX) summarized the nongenetic animal models for UV-induced melanomagenesis, in which mice, the Mexican opossum *Monodelphis domestica*, or *Xiphophorus* hybrid fish are exposed to UV alone or in combination with chemical carcinogens. The best-characterized genetic mouse model of melanoma was developed by Lynda Chin and coworkers at the Dana-Farber Cancer Center, Boston, MA. Mice with the deleted *INK4A* locus were crossed with transgenic animals expressing the *ras* oncogene under a tyrosinase promoter. The progeny developed melanoma with a high frequency. An additional mouse melanoma model was discussed by Suzie Chen (Rutgers University, Piscataway, NJ). Melanoma spontaneously developed after insertion of an irrelevant transgene. The session demonstrated that the human *in vitro/in vivo* orthotopic model is best suited for studies on early melanocytic lesions and tumor-stroma interactions, whereas the current mouse genetic models are best suited to characterize progression of melanoma to an aggressive phenotype. The further development of mouse genetic models for studies in early melanocytic lesions will be challenging because in mice, melanocytes are located predominantly deep in the hair follicles and in the dermis but rarely in the epidermis. In contrast, melanocytes in humans are predominantly located within the basal layer of the epidermis. A transgenic mouse model developed by Glenn Merlino and coworkers at the National Cancer Institute, Bethesda, MD uses hepatocyte growth factor under a metallothionein promoter to retain melanocytes close to the epidermis. UV irradiation of newborn mice resulted in increased melanoma development. This model shows promise for UV-induced melanomagenesis. Additional refinements of genetic melanoma models are needed to optimally mimic the conditions of melanoma development and progression in humans. Such models are expected to play an increasingly important role for investigations in melanoma prevention, diagnosis, and therapy.

The session on tissue environment and melanoma, chaired by Ruth Halaban (Yale University, New Haven, CT) dealt with UV-induced signaling, receptor tyrosine kinase signaling, and humoral and cell-mediated host responses. Ze'ev Ronai (Mt. Sinai School of Medicine, New York, NY) outlined how melanoma cells signal after UV or ionizing irradiation and how knowledge of the signaling pathways can help in developing new strategies for overcoming the notorious radiation and chemoresistance of melanoma cells. In contrast to normal melanocytes, melanoma cells produce a variety of cytokines and growth factors for autocrine and paracrine stimulation. Ruth Halaban listed the major receptor tyrosine kinases that are active in the melanocyte system, including those for basic fibroblast growth factor, hepatocyte growth factor, stem cell factor, and insulin-like growth factor signaling. Cell cycle dysregulation appears to play a major role in melanoma, particularly through the RB and cyclin D pathways.

Melanoma cells are highly immunogenic in patients, eliciting both humoral and cell-mediated immune responses. In fact, melanoma has been the prime model among all of human cancers for studying the immune response against cancer cells because of the relative ease of culturing normal and malignant melanocytes. Soldano Ferrone (Roswell Park Cancer Institute, Buffalo, NY) pointed to the potential dysregulation of the cell-mediated immune response in melanoma patients, which may be attributable to down-regulation or loss of HLA class I molecules. The latter abnormalities are caused by mutations in the genes encoding the HLA class I subunits and/or by defects in the

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components of the antigen-processing machinery. Francesco Marincola (National Cancer Institute, Bethesda, MD) analyzed the phenotypes of melanoma cells by global gene expression before and after cytokine therapy to develop better prognostic criteria for cytokine and vaccine therapies. New antigens are currently being identified that are suitable for eliciting both humoral and cell-mediated immune responses. The strategies used in these studies that may lead to the development of new vaccine targets were summarized by Yao-Tseng Chen (Cornell University, New York, NY) and Dorothee Herlyn (The Wistar Institute, Philadelphia, PA). Genes associated with pigmentation appear particularly immunogenic. Peptides derived from pigment-related and -unrelated proteins are being used for active immunization, either together with adjuvants or coupled to autologous dendritic cells that are propagated *in vitro*. Antigen-specific T cells from melanoma patients can also be expanded *in vitro* and then reinfused to the same patients in an adoptive immunotherapy strategy, as demonstrated by Cassian Yee (Fred Hutchinson Cancer Center, Seattle, WA). It became obvious from this session that rapid progress is being made in identifying new biological and immunological targets for melanoma therapy. However, each strategy has its own challenges to overcome. New *in vivo* imaging techniques, as demonstrated by Dorothea Becker (University of Pittsburgh, Pittsburgh, PA), should help in validating the biological significance of selected targets.

The final session on melanoma progression and stroma was chaired by David Fisher (Dana-Farber Cancer Institute, Boston, MA). It dealt with tumor matrix, motility and invasion, and transcriptional regulation of growth. Peter Brooks (New York University, New York, NY) has identified epitopes in collagen that are potential targets for therapy because only melanoma-derived enzymes expose them. Matrix metalloproteinases, the extracellular matrix, and adhesion receptors form functional units that are critical for tumor progression (Yves DeClerk, University of California, Los Angeles, CA). Melanoma cells may also develop properties that resemble those of endothelial cells. Mary Hendrix and coworkers have identified channels that are devoid of endothelial cells but still allow blood flow. David Fisher outlined the major transcription factor systems. For melanocyte development, the MITF transcription factor appears most critical. In melanoma, there are apparently three major transcription factor systems. The activating transcription factor-2 and nuclear factor κB pathways are conferring signals for apoptosis resistance (Ze'ev Ronai), and AP-2 regulates expression of critical cell surface receptors for melanoma cells such as c-KIT and MUC18/M-CAM (Menashe Bar-Eli, M. D. Anderson Cancer Center, Houston, TX). This session highlighted that knowledge from melanocyte development helps to better understand the transcriptional dysregulation in melanoma cells.

The group then addressed critical issues in the melanoma field and made recommendations to strengthen it and advance scientific knowledge to stem the dramatic increases in melanoma incidence in recent decades and the continuing lack of effective therapies. To make the melanoma research field more attractive for new and established investigators and increase cooperation among established investigators, the overall research base should be strengthened by:

(*a*) Increase communications among melanoma researchers. The melanoma research community has had few meetings in recent years that provide a forum for discussions among researchers. Regular meetings should bring together basic and clinical investigators to foster collaborations in translational research. More frequent meetings should also encourage scientists from related fields to attend. In turn, the latter should be encouraged to present new approaches and technologies.

(*b*) Develop tissue banks and make them accessible to investigators through national networks. Whereas metastatic lesions are relatively easily accessible, it has become increasingly difficult in recent years to accrue biologically early lesions for experimental studies. The scarcity of tissues is hampering progress in the field, particularly in the development of new diagnostic markers for intermediate lesions between benign and malignant. Advocacy groups should be contacted to obtain the cooperation of melanoma patients, who should be encouraged to donate lesions that are not needed for diagnosis.

(*c*) Exchange data from global gene expression analyses of cells from melanocytic lesions of different stages of tumor progression including melanocytes from normal skin. The databases should be accessible to collaborating laboratories and should include analyses of DNA, RNA, or protein samples. Genes that are silenced through methylation should be identified. Such studies should allow a molecular fingerprint of the dynamics of tumor development and progression.

(*d*) Develop EST libraries from melanocytic cells for expression analyses, sequence the clones, and secure their distribution to interested scientists for global gene expression and functional genomics.

(*e*) Share research tools such as antibodies, cDNA, cell lines, vectors, and animal models.

(*f*) Foster advocacy groups who could help to increase research funding at federal and philanthropy levels for basic and translational research.

Scientific challenges continue to hamper the melanoma research field. The group pointed to several priority areas, including:

(*a*) Find a molecular basis for melanoma susceptibility. More efforts are required to find the genetic basis for both familial and sporadic melanoma. The melanoma field lags behind other tumor types in identifying the structural abnormalities in melanoma cells that are commonly found in malignant lesions. Early progress has already been made in identifying genes associated with increased risk for melanoma, but much work remains to be done. A better understanding of the pigmentation pathways should also help in developing a molecular base for risk assessment.

(*b*) Establish the biological and genetic basis for nevus development and determine genetic abnormalities in dysplastic nevi that may serve as markers for detection.

(*c*) Determine host- and tumor-derived factors that regulate progression from the radial to the vertical growth phase of primary melanoma. Investigate the cross-talk between tumor cells and the stromal environment and develop therapeutic approaches that target not only tumor cells but also endothelial cells and fibroblasts. Determine the role of inflammatory and immune cells for disease progression.

(*d*) Identify checkpoint genes in the melanocyte system and the signaling pathways that control growth, apoptosis, differentiation, and senescence.

(*e*) Delineate the roles of UVB *versus* UVA.

(*f*) Develop robust animal models of early, intermediate, and advanced melanocytic lesions that can help to develop new strategies for prevention and therapy.

(*g*) Investigate the clonality of nevus and melanoma formation and the extent of molecular and phenotypic heterogeneity.

(*h*) Investigate the biology of micrometastases and the factors that control their dormancy or progression.

(*i*) Identify new targets for active and passive immunotherapy of melanoma. Delineate strategies to augment antigen-specific immunity.