Meeting report: Melanoma Initiative Symposium

The Inaugural Mini-Symposium on Melanoma research at Case Western Reserve University, University Hospitals, Cleveland Clinic, and VA Medical Center was held at the Wolstein Research Building Auditorium on March 11, 2013. The symposium was organized by Dr. Nikki Harter (Department of Biochemistry/CWRU), Dr. Barbara Bedogni (Department of Biochemistry/CWRU), and Dr. Meg Gerstenblith (Department of Dermatology/UHCMC). The conference consisted of 13 oral presentations, and talks were arranged into five oral sessions according to their scientific content. A social event consisting of a box dinner provided the opportunity for basic researchers and clinician scientists to develop new collaborations on melanoma research.

Dr. Stan Gerson, Director of Case Comprehensive Cancer Center and Seidman Cancer Center, welcomed all of the participants to the conference and expressed his enthusiasm in developing a robust research program on melanoma. Dr. Kevin Cooper, Chair of the Department of Dermatology and Director of the Skin Disease Research Center, gave the closing remarks and discussed how this meeting highlighted the importance of integrating clinical expertise with unprecedented advances in knowledge from basic scientists in the quest of understanding melanoma and improving clinical outcomes.

Session 1. Epidemiology
Dr. Jeremy Bordeaux (Department of Dermatology/UHCMC) reported that patients have an increased risk of developing a melanoma following the diagnosis of another form of cancer, e.g., breast cancer, prostate and thyroid. He also reported on ways of educating patients in identifying melanoma through skin self-examination. Dr. Bordeaux concluded, with his own study, that the original Breslow depth of transected melanomas without residual tumor or re-excision, accurately predicts prognosis and survival.

Dr. Meg Gerstenblith (Department of Dermatology/UHCMC) discussed the fact that melanoma is an etiologically heterogeneous disease and how advanced molecular techniques might allow for new classifications of melanoma. She discussed her plan to establish a melanoma database/repository linked to clinical information (histopathologic characteristics, phenotypic, epidemiological, and current clinical data) on the patients enrolled. Dr. Gerstenblith also reviewed her plan on how she and her collaborators at NIH intend to use array-comparative genomic hybridization (array-CGH) on formalin-fixed paraffin-embedded (FFPE) melanoma tissue to identify clinically relevant subtypes of melanoma, all in the effort to guide the selection of therapies for patients with this disease.

Session 2. Genetics and Environmental effects
Dr. Daniel Lindner (Taussig Cancer Institute/CCF) opened the session by discussing how pharmacological intervention might reverse epigenetic aberrations such as promoter hypermethylation in melanoma, with the possibility of interfering with tumor progression. Dr. Lindner reported that when established melanoma cell lines were treated with 5-Aza-deoxycytidine (5-Aza-dC), a chemical that ultimately results in the depletion of DNA methyltransferase (DNMT1), he and his collaborators observed a reversal in the hyper-
methylation of a promoter regulating thrombospondin-1 (TSP1), a known inhibitor of angiogenesis. In a mouse model receiving 5-Aza-dC, TSP1 was significantly increased, inhibiting new blood vessel formation, and suppressing tumor growth. Finally, Dr. Lindner used primary human nevi and melanoma tissue to demonstrate that the promoter of TSP1 had a higher frequency of methylation in the latter samples.

Dr. Nikki Harter (Department of Biochemistry/CWRU) discussed her evidence of simulated sunlight (ssUVR) effecting a differential response in the expression of microRNAs (miRNAs) in melanocytes of healthy individuals and those with a history of melanoma. Intense UVR (UVA + UVB) is a major environmental risk factor for cutaneous melanoma, and a growing body of experimental evidence now supports this notion. Dr. Harter reported on new experimental approaches to examine, for the first time, the potential effects of ssUVR on melanocytes while in their in vivo microenvironment. She identified several groups of UVR-responsive miRNAs that were predicted to target genes involved in a variety of pathways, including DNA repair. Most notably, these miRNAs were consistently down-regulated in healthy individuals, but up-regulated in the melanoma patients.

Dr. Thomas LaFramboise (Department of Genetics/CWRU) discussed the mitochondrial mutational landscape in melanoma. Somatic mutations in mitochondrial DNA (mtDNA) have long been proposed to drive the progression of human malignancies. Using paired-end massively parallel sequencing techniques, Dr. LaFramboise interrogated mtDNA isolated from the blood of 18 melanoma patients to identify potential somatic mutations. Among the 18 samples, 13 were found to carry somatic mutations in the mtDNA. Most of these mutations were scattered, but others were concentrated in genes encoding cytochrome oxidase subunits 1, 2, and 3. These mutations are also commonly found in the mtDNA of breast cancer patients, thereby suggesting that they may be biologically relevant.

Session 3. Cell Signaling

Dr. John Letterio (Department of Pediatrics/CWRU/UHCMC) opened the session by discussing the potential role of the cyclin-dependent kinase, Cdk5, in melanomagenesis. Cdk1 and Cdk2, and the two cell cycle-independent cyclin kinases, Cdk5 and Cdk9, are an attractive class of molecules for the development of inhibitors since they are expressed at high levels in advanced melanoma cell lines. Dr. Letterio reported that Cdk5 and its cognate partner p35 are invariably and constitutively expressed in both melanoma cell lines and melanoma samples. He also provided evidence that selective or specific inhibition of Cdk5 in melanoma cell lines and in melanoma xenographs dramatically suppresses their viability, mainly by modulating AKT signaling. Finally, Dr. Letterio discussed that the abrogation of Cdk5 activity restores an inhibitory response to TFG-β in melanoma.

Dr. Bingcheng Wang (Department of Pharmacology/CWRU) reported on the Eph/ephrin system, specifically EphA2, and its role in the regulation of melanoma invasion and metastasis. EphA2, which is known to participate in various developmental processes, belongs to the large Eph family of receptor tyrosine kinases and is highly expressed in a number of melanoma cell lines. Moreover, EphA2 can regulate vascular mimicry in melanoma and its depletion inhibits tumor growth in vivo. Dr. Wang presented data from his prior work showing that the corresponding ligand Ephrin-A1 of EphA2 could inhibit the growth of primary keratinocytes from EphA2+/+ but not EphA2/-/- mice. He also reported that EphA2 knockout mice are more
susceptible to 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA) two-stage chemical carcinogenesis compared to wild-type mice, and that EphA2 can stimulate tumor cell migration and invasion in a ligand independent manner. Finally, Dr. Wang is now investigating the effects of doxazosin on melanoma cell lines. Doxazosin is a novel EphA2 agonist which can inhibit chemotactic cell migration of multiple cancer cell types. Evidence for this effect was presented.

Dr. Shigemi Matsuyama (Department of Medicine/CWRU) talked about INF \(_\gamma\) Receptor 2 (IFNgR2) and its potential role in conferring apoptotic resistance in melanoma cells. Dr. Matsuyama’s research focus has been on Bax, a pro-apoptotic protein which when activated, triggers mitochondrial outer membrane permeabilization (MOMP) and release of proapoptotic factors, such as cytochrome c. Dr. Matsuyama has evidence that IFNgR2 can bind directly to Bax and thereby inhibit its activity in mediating apoptosis. He also demonstrated that IFNgR2 is expressed at high levels in melanoma cells, and when subjected to shRNA-mediated knockdown, apoptosis is then induced in these cells. Dr. Matsuyama hypothesizes that IFNgR2 may have a ligand independent activity as a Bax inhibitor in cells and that this may account for melanoma’s resistance to apoptosis.

Session 4. Immunology

This session began with a talk by Dr. Julian Kim (Department of Surgery/CWRU/UHCMC) who discussed his use of adoptive immunotherapy in the treatment of metastatic melanoma, a highly promising approach. He showed how it was possible to harvest melanoma-specific T cells from the melanoma-draining lymph nodes (MDLN) of patients in stage 111, grow them ex vivo, and then select the cells for transfer back into the host. He presented evidence that MDLN cells cultured in IL-2 or IL-2/IL-7 resulted in T-cell expansion and that when cultured for 14 days could mediate specific apoptosis after their incubation with melanoma cell lines. Moreover, addition of an antibody specific for VEGF to MDLN cultures resulted in T cells having significant increases in both intracellular INF \(_\gamma\) and TNF\(_\alpha\). Finally, evidence for the improved survival of mice bearing melanoma xenographs treated with MDLN cells grown with VEGF blockade was presented. A phase 1 study using ex vivo-activated lymph node lymphocytes in advanced malignant melanoma patients is now underway.

Dr. Thomas McCormick (Department of Dermatology/UHCMC) highlighted the infiltration of INF-\(\gamma\) secreting macrophages in human melanoma samples by immunofluorescence confocal microscopy. INF\(_\gamma\) from recruited macrophages in UVB-irradiated neonatal mice are known to stimulate melanocyte proliferation and migration. Using frozen melanoma tissue taken from a total of ten patients, Dr. McCormick, and colleagues showed by immunofluorescence the presence of INF\(_\gamma\), lymphocytes and macrophages in each of the respective samples. Moreover, macrophages that had infiltrated the skin was shown to produced INF\(_\gamma\). Finally, Dr. McCormick presented data on three genes (CXCR3, PSTAT-1, MIG/CXCL9) known to be induced by INF\(_\gamma\), each of which could be visualized by immunofluorescence in the melanoma samples.

Session 5. Therapeutics/Drug discovery

Dr. Barbara Bedogni (Department of Biochemistry/CWRU) opened the final session by describing experiments that demonstrate the feasibility of targeting the developmental pathways of Notch and the ERBB family of tyrosine kinase receptors (ERBB1, 2, 3, and 4) for melanoma
therapy. Notch is involved in the development of melanocytes, and Dr. Bedogni has recently identified Notch1 in the up-regulation of Neuregulin 1 (NRG1), which in turn activates ERBB2/ERBB3 signaling, an axis which is key to melanoma survival and growth. In this session she described the effects of known Notch and ERBB inhibitors, DZB and LAP (lapatinib) respectively. Each can modestly reduce melanoma viability, but when added together, their effect becomes more dramatic, whether the melanoma cell lines are wild-type or mutant for BRAF. Moreover, treatment of cells with both inhibitors affected the phosphorylation of AKT and the expression of NF-κB. Finally, Dr. Bedogni reported that both inhibitors could effectively inhibit tumor growth in xenograph models when compared to untreated control.

Dr. Chad Zender (Department of Otolaryngology/CWRU/UHCMC) discussed recent advances in sentinel lymph node (SLN) localization in head and neck melanoma. Sentinel lymph node biopsy is a minimally invasive method to detect the presence of nodal micrometastases and represents a new model for the initial management of patients with stage I and II melanoma. Dr. Zender reported on techniques for identifying SLNs (e.g. injection of radio-colloid plus blue dye or blue dye alone), but presented evidence for using single-photon emission computed tomography/computed tomography (3-D SPECT CT). This technique allows for more accurate surgical planning and in identifying the anatomic location of SLNs in the head and neck region where the primary tumor is close to the regional basin or basins.

Dr. Henry Koon (Department of Medicine/CWRU/UHCMC) gave an important overview on the state of approved and developmental therapeutics for melanoma. He discussed the shortcomings of current melanoma therapies, particularly in the use of vemurafenib and ipilimumab, which selectively inhibits a BRAF mutation and blocks the cytotoxic T-lymphocyte antigen-4 pathway from inhibiting T cell activation, respectively. In most cases, resistant mechanisms quickly develop leading to relapse and subsequent patient mortality. Dr. Koon also alluded to the necessity of combining therapeutic modalities, which may increase the percentage of responding patients, ultimately by attacking different paths of tumor escape. Finally, Dr. Koon suggested that radiotherapy might still be required in adjuvant settings to reduce local recurrence rates.

Looking Forward

In summary, the high quality of the presentations that were given at this meeting not only highlighted the fact that melanoma remains a difficult and highly aggressive type of skin cancer, but how its development is influenced by genetic factors of the individual subject, the genetic/epigenetic composition of the disease, and environmental influences on its initiation. Topics of discussion such as immunology, new cell signaling pathways, and environmental effects provided a broad platform for communication and collaboration for anyone who is interested in melanoma research. Given the great success of this meeting, it is anticipated that a small number of groups comprised of basic researchers and clinicians with common interests in specific areas of melanoma research will now be instituted, with an emphasis on the development of new therapeutic approaches and the procurement of outside funding. Participants would include investigators from Case Western Reserve University, University Hospitals, Cleveland Clinic, and the VA Medical Center. An organization of such is forthcoming and will be under the sponsorship of the Case Comprehensive Cancer Center.