Recent Advances in Melanoma and Melanocyte Biology

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Numerous recent articles, many published in the JID, have brought about new discoveries and insights into melanoma and melanocyte biology. A quick synopsis of several important articles is outlined below.


MELANOMA IMMUNOTHERAPY—SEARCH FOR MORE PLAYERS

Recent advances in immunotherapy have significantly prolonged the overall survival of patients with melanoma. However, benefit from immunotherapeutic drugs is limited only to patients who achieve durable responses. Several recent articles/reports featured potential mechanisms behind the lack of response in immunotherapy.

Previously, TIGIT (T-cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motif domains) had been shown to be an immune checkpoint molecule that can limit CD8+ T-cell-dependent antitumor responses in a similar manner to cytotoxic T-lymphocyte-associated protein 4 and programmed cell death protein 1 (Johnston et al., 2014). Subsequently, it was also reported that TIGIT blockade increases melanoma-specific cytotoxic T lymphocytes (Chauvin et al., 2015). Inozume et al. (2016) demonstrated that TIGIT blockade enhances the melanoma-specific cytotoxic T lymphocyte response specifically in the effector phase using an in vitro coculture assay. These studies also strongly suggest that coblockade of TIGIT and PD-1 may enhance antitumor CD8+ T-cell responses in patients with melanoma, because TIGIT-positive cells often coexpress PD-1 (Inozume et al., 2016).

A report from Gulati et al. (2016) described another therapy that could potentially be combined with anti-PD-1 blockade. Diphenylcyclopropenone (DPCP) has been used in patients with melanoma as a sensitizing agent to induce tumor regression. Gulati et al. analyzed five patients showing partial or complete melanoma metastasis regression after DPCP treatment to explore the mechanisms involved in immune-mediated tumor regression. Their analysis revealed that DPCP treatment induced extensive immune cell infiltrates, including T cells, myeloid dendritic cells, and macrophages. T helper type 1-related genes were upregulated in DPCP-applied regions compared with pre-DPCP metastasis. Interestingly, PD-1 expression was also significantly increased in DPCP-applied regions, suggesting the possibility that DPCP and anti-PD-1 therapies may complement each other.

Dendritic cells are the main antigen-presenting cells that prime cytotoxic T lymphocyte response; however, they are also known to contribute to disease progression in many cancers. It has been proposed that tumor cells can suppress dendritic cell function and/or recruit immune-suppressive dendritic cells. A study from Frue’s group characterized tumor-infiltrating dendritic cells (TIDCs) obtained from early to late stages of the murine melanoma model (Nakahara et al., 2016). Unexpectedly, their analysis showed that early TIDCs express immunoinhibitory molecules, but that later TIDCs present an immunostimulatory phenotype during tumor growth. This observation is in line with findings from other studies that suggest that TIDCs become maturated, immunostimulatory antigen-presenting cells under a certain tumor microenvironment. However, the molecular mechanisms behind this phenotype switch in TIDCs remain to be elucidated.

Current immunotherapy approaches for melanoma are mainly focused on directly modifying the activity of adaptive immune cells. However, recent research advances also highlight the importance of the innate immune system in limiting cancer progression (Liu and Zeng, 2012). Retinoic acid-inducible gene I, a pattern recognition receptor in the cytosol of mammalian cells, functions as a sensor for viruses and triggers a type I IFN-driven antiviral immune response. A previous study showed that short double-stranded RNA fragments carrying an uncapped 5’-triphosphate moiety (ppp-RNA) can be utilized to silence an oncogene and simultaneously activate the immune response (Pocek et al., 2008).

Matheis et al. (2016) tested a similar approach to activate retinoic acid-inducible gene I and to silence the urokinase-type plasminogen activator receptor oncogene by bifunctional ppp-small interfering RNA. Treatment with ppp-urokinase-type plasminogen activator receptor stimulated a systemic immune response in xenograft mice and reduced tumor growth. Because urokinase-type plasminogen activator receptor expression is strongly upregulated in melanoma cells with acquired resistance to BRAF and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase inhibitors, and those resistant cells were effectively killed by ppp-urokinase-type plasminogen activator RNA (ppp-MAPK/ERK siRNA).

Abbreviations: CNV, copy number variation; DPCP, diphenylcyclopropenone; MAPK, mitogen-activated protein kinase; MPA, multiple primary melanoma; sFRP2, secreted frizzled-related protein 2; SPM, single primary melanoma; TIDC, tumor-infiltrating dendritic cell; TIGIT, T-cell immunoreceptor with Ig and ITIM domains

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Received 2 November 2016; accepted 3 November 2016; corrected proof published online 12 January 2017
activator receptor, the authors propose that this approach has the potential to help overcome the therapy resistance of melanoma.

**MELANOCYTE, MELANOMA, AND UV RADIATION**

Melanocyte biology is strongly influenced by UV light. UV radiation is considered a major carcinogen for skin cancer, but it is also known to have tumor-promoting effects in already established tumors. Several intriguing papers on the impact of UV light on melanocyte/melanoma biology emerged recently.

Kamenisch et al. (2016) reported a novel mechanism for UV-induced melanoma progression. The authors demonstrated that UVA induces the Warburg-like effect and changes the metabolism of melanoma cells. They showed that UVA irradiation increased glucose uptake and lactate production, which promotes melanoma cell invasion through upregulation of matrix metalloproteinases. Their finding is likely more relevant to early metastasis events, because the UVA-induced invasion was induced more robustly in primary, indolent melanoma cells compared with metastatic melanoma cells. The study also suggests that sun protection is critical for not only preventing transformation of melanocytes, but also for preventing the invasion of already transformed cells.

Earlier this year, Kim et al. (2016) showed that a Wnt signaling modulator sFRP2 (secreted frizzled-related protein 2) is involved in pathology of UV-induced hyperpigmentation disorders such as melasma and solar lentigo. sFRP2 is increased in UV-induced hyperpigmented skin. Using gain-of-function and loss-of-function experiments, the authors demonstrated that sFRP2 activates β-catenin signaling and induces pigmentation through microphthalmia-associated transcription factor upregulation in normal melanocytes. This induction of sFRP2 in UV-irradiated skin is particularly relevant considering another elegant study from Kaur et al. (2016), in which the authors showed that sFRP2, which is increased in aged skin, plays a role in melanoma metastasis and therapy resistance. In contrast to the Wnt activator role in normal melanocytes, sFRP2 decreases β-catenin and microphthalmia-associated transcription factor in expression melanoma cells, suggesting that sFRP2 has dual functions as an activator and inhibitor of the canonical Wnt pathway in a context-dependent manner.

Melanocytes slowly proliferate and are resistant to UV-induced apoptosis. Their longevity in the skin may be associated with a risk of cumulative genetic damage, which in turn may lead to transformation. Bin et al. (2016) described a novel mechanism behind the resistance to UV-induced apoptosis in melanocytes. They found that UVB increases the secretion of extracellular vesicles from melanocytes. The melanocyte-derived extracellular vesicles contain fibronectin, which protects the pigment cells from UV-induced apoptosis. Fibronectin is a critical molecule in cell adhesion and invasion. It binds to integrin receptors and plays a key role in embryogenesis, tissue repair, and cancer. A new study from Fedorenko et al. (2016) showed that fibronectin-integrin signaling mediates BRAF inhibitor resistance in melanoma cells by maintaining the expression of the pro-survival protein Mcl-1. This fibronectin-integrin-Mcl-1 axis is also required for resistance to anois (Boisvert-Adamo et al., 2009), suggesting that melanocytes and melanoma cells share this survival mechanism during stress conditions such as UV irradiation, drug treatment, or cell detachment. It is notable that several recent studies have also highlighted the biological role of extracellular vesicles (i.e., “exosomes”) in melanoma invasion and metastasis (Dror et al., 2016; Peinado et al., 2012). Further studies are needed to elucidate whether and how UV-induced extracellular vesicles are relevant with early transformation events in melanocytes.

**MELANOMA EPIDEMIOLOGY AND GENETICS**

Numerous studies over the years have monitored and tracked the burden of melanoma in a retrospective fashion. The results have all shown a steady increase in the rates of melanoma over the past few decades. But how about the future? Health care policy watchers are more interested in the behavior, and thus economics, of diseases going forwards than they are about the distant past. David Whiteman’s group in Queensland, Australia, projected the growing incidences of melanoma through 2031 around the world and found some surprising results (Whiteman et al., 2016). The authors used three decades of cancer registry data (1982–2011) from six populations including the United Kingdom, Sweden, Norway, Australia, New Zealand, and the United States. They then constructed age-period-cohort models to examine and compare current observable trends with projected trends. In all countries, the highest age-specific invasive melanoma rates were in the elderly (>80 years), and this is projected to continue because the proportion of individuals more than 80 years will also increase. For those actuarial enthusiasts, there are plenty of graphs and figures showing growth over time in various countries. Perhaps the most striking estimate is that among US whites, the number of new cases of melanoma will rise from approximately 70,000 cases in 2007–2011 to 116,000 in 2026–2031. The bulk of this increase is thought to be attributable to rising age-specific rates and to population growth and aging. It is worth mentioning that melanoma mortality may be dramatically changing in the opposite direction. There was a time when stage IV disease was essentially equated with lethality. With the advent of contemporary molecular and immunologically therapeutics, the parallel incidence and mortality rates observed in the past may start diverging.

Another interesting epidemiologic study from Queensland comes from Kiara Khosrotehrani’s group (Youlden et al., 2016). These investigators looked at the survival of patients who have had a single primary melanoma (SPM) versus those who have had more than one. These patients with multiple primary melanoma (MPM) account for 1–10% of all cases when reported in the literature. Traditional teaching suggests that there is a “survival bias” when looking at individuals with MPM because these patients have had a longer time to develop more than one melanoma. These investigators undertook an innovative new statistical approach called “delayed entry” to mitigate against the MPM “survival bias.” After adjusting for known prognostic factors, the hazard ratio of death within 10 years from melanoma was two times higher for those with two melanomas (hazard ratio = 2.01, 95% confidence interval = 1.57–2.59; P < 0.001) and nearly three times higher when three melanomas were diagnosed (hazard ratio = 2.91, 95% confidence interval = 1.64–5.18; P < 0.001) compared with people with a single melanoma. Other studies have found contradictory
findings (i.e., patients with MPM did better than those with SPM) perhaps due to the methodology of analysis as alluded to above. Also, when compared with the only melanoma in the SPM cohort, the first melanoma for patients with three melanomas was more likely to occur on the trunk, have nodular morphology, and be at least 2 mm in thickness; the second and third melanomas were not characteristically different from the only melanoma in the SPM group. As the authors posit, it is thus possible that individuals who are prone to develop MPM are more likely to form aggressive tumors and thus exhibit a diminished overall survival. Although this hypothesis remains to be tested, the results of this study do have clinical implications for the follow-up surveillance of patients with SPM and MPM.

Lastly, copy number variations made an entry into the domain of melanoma susceptibility. To date, the prevailing evidence indicates that common single nucleotide polymorphisms, either coding or noncoding, account for sporadic melanoma risk, whereas rare missense or deleterious mutations underpin familial risk. What about copy number variations (CNVs)? CNVs have been shown to be disease modulating by altering gene dosage, disrupting coding sequences, or perturbing long-range gene regulation. Investigators from the National Cancer Institute studied the role of CNVs in melanoma prone families (Shi et al., 2016) by comparing structural alterations in affected and unaffected family members and spouse controls. The global burden of overall CNVs (or deletions or duplications separately) was not significantly associated with disease suggesting that most CNVs represent no- or low-risk polymorphic changes. However, when rare CNVs in 35 known melanoma genes were assessed, there was a 1.3 Mb CNV on chr 1q42 and a 10 kb CNV on chr 9p21, which were found in one and five affected individuals, respectively. Beyond the candidate approach, the authors also examined rare CNVs that cosegregated within families. Using this metric, four CNV regions were found: chr 2q22.1 loss (112 kb), chr 3p12.2 gain (480 kb), chr 8q24.3 loss (5–15 kb), and 10q23.33 gain (260 kb). Expression changes for genes within these affected regions generally followed the patterns of gains and losses. This is the first comprehensive look at germline CNVs for melanoma. Although the data are provocative, larger cohorts are needed. Unlike the weight of evidence for missense and harmful mutations, the case for CNV as a bona fide genomic risk factor needs further substantiation.

**MELANOMA TRIALS**

Although the JID is not typically a venue for advanced cancer trials, it is still worthwhile for our readership who have an interest in melanoma therapeutics to peruse two key trials that have emerged in 2016. Eggermont et al. (2016) led the EORTC in a trial testing the efficacy of ipilimumab (anti-CTLA4) for patients for stage III melanoma. Before this study, ipilimumab had already been approved for stage IV disease; the question to be asked by this study is whether adjuvant treatment can improve survival of stage III patients who are at high risk for relapse. With a median follow-up of 5.3 years, the 5-year recurrence-free survival rates were 40.8% in the ipilimumab group and 30.3% in the placebo group ($P = 0.001$), and the 5-year overall survival rates were 65.4% in the ipilimumab group and 54.4% in the placebo group. Adverse events were far more significant in the ipilimumab group with five treatment-associated deaths. The FDA did approve the use of ipilimumab for stage III adjuvant treatment although the uptake among oncologists remains to be seen. Beyond ipilimumab, the age-old standard for adjuvant stage III treatment has been IFN-$\alpha$.

Sentinel lymph node biopsies allow physicians to identify regional metastases before the development of bulky nodal relapse. It is performed to varying degrees around the world though a survival benefit has never been shown (Morton et al., 2014). For those patients with microscopic disease in the sentinel lymph node, removal of all nodes in the entire nodal basin is standard of practice. However, the benefit of this additional morbid procedure has never been shown. German investigators set out to precisely ask this question in a recent surgical trial designated DeCOG-SLT (Leiter et al., 2016). Of 5,547 patients who underwent a sentinel lymph node biopsy, 1,269 were found to be positive, and 473 of these patients with microscopic disease were eventually randomized to observation versus completion lymph node dissection (i.e., removal of the rest of the lymph nodes in the basin). The distant metastasis-free survival at 3 years was not different between the two groups (77.0% vs. 74.9%, respectively). As expected, surgical complications, including lymphedema and infection, were higher in the dissection group compared with the observation group. This exact subject matter is under trial in the United States under the Multicenter Selective Lymphadenectomy Trial II trial, which has yet to mature for publication. If, in fact, these results are replicated and found to be sufficiently powered, it would greatly alter surgical practices because the rather morbid procedure of a subsequent lymph node dissection may be avoided.

**TARGETS FOR SIGNALING THERAPY**

Signaling therapy of melanoma emerged after the BRAFV600E mutation was discovered in 2002 with the first drug approved in 2011, a remarkably short time span considering how long drug development often takes. But soon after approval came the awareness of resistance, which the melanoma cells developed after continuous therapy. The mechanisms of resistance to BRAF or combinations of BRAF and MAPK/extracellular signal-regulated kinase inhibitors are multifold and may vary between tumors and even within tumors. Beaumont et al. (2016) point to the importance of the cell cycle in intrinsic resistance to signaling inhibitors, even alkylating agents. Cells that do not proliferate are generally refractory to inhibitors of signaling pathways associated with growth. As the cells are in a survival and no-growth mode, they often show increased mitochondrial metabolic activity, are high in autophagy, and may even express markers of senescence. As the stress from therapeutics eases, cells adjust again quickly and reenter the cell cycle. The MAPK signaling pathway, which is associated with proliferation, remains the most important to target. Besides BRAF, which is mutated in 50% of melanomas, neuroblastoma RAS viral oncogene homolog is mutated in another 25%. Two reviews in 2016 teach us about the intricacies of NRAS. Posch et al. (2016b) guide us through the intricacies of the complex signaling for NRAS in melanoma, whereas Halaban and Krauthammer (2016) explain genetic diseases associated with germine RAS
mutations and point to clinical symptoms that may also occur in patients whose melanomas have somatic NRAS mutations. Fortunately, melanomas do not carry the most aggressive V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mutations, because these lead, like in pancreatic cancers, to highly aggressive disease progression. Direct drug targeting of any of the RAS mutations is still elusive, but Posch et al. (2016a) discovered an indirect target. The casein kinase 2 alpha kinase is upregulated in NRAS-mutant melanomas and can be successfully targeted with a small molecule inhibitor. The MAPK pathway is also activated in patients’ melanomas that do not carry BRAF or NRAS mutations because most growth factor receptors transmit their signals through this pathway to stimulate proliferation. An example is the EGFR family member ERBB3. Synchronous targeting of both the MAPK pathway and Notch signaling strongly inhibited melanoma growth (Zhang et al., 2016), and this strategy may also affect cancer stem-cell-like populations that are upregulated by Notch signaling (Kumar et al., 2016).

Besides the MAPK signaling pathway, melanoma cells have frequent alterations in the phosphoinositide 3-kinase/acutely transforming retrovirus AKT8 in rodent T-cell lymphoma survival pathway that all lead to constitutive activation. A prominent example is the tumor suppressor gene phosphatase and tensin homolog that controls (suppresses) PI3K/acutely transforming retrovirus AKT8 in rodent T-cell lymphoma activation. Although mutations/deletions are only present in one quarter of melanomas, the PTEN gene is silenced through hypermethylation in the majority of melanomas. Silenced PTEN is also an outstanding biomarker predicting survival of melanoma patients (Roh et al., 2016). The research field continues to discover new targets and drugs for therapy. The challenge will be to integrate new treatment strategies for optimal synergy with the most successful current therapies.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**REFERENCES**


