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.

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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 (Poeck 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

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Abbreviations: CNV, copy number variation; DPCP, diphenylcyclopropenone; MAPK, mitogen-activated protein kinase; MPM, 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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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 medicates 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 anoikis (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 Kiarash 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