So You Can Teach Old Fibroblasts New Tricks

Amaya Virós, Maria Romina Girotti, and Richard Marais

Summary: New data show that as dermal fibroblasts grow old, they increase their secretion of the WNT antagonist sFRP2 to drive melanoma cell metastasis. sFRP2 suppresses β-catenin and MITF signaling in melanoma cells, downregulating the redox regulator APE1, making melanoma cells more sensitive to oxidative stress and driving resistance to BRAF inhibitors. Thus, the aging microenvironment in elderly patient skin activates a signaling pathway that drives more aggressive melanoma cell behavior. Cancer Discov; 6(6); 581–3. © 2016 AACR.

Cancer is more common in the elderly, and in melanoma, incidence and mortality continue to rise in this population, particularly in those older than 65 (1). Before the discovery of the revolutionary targeted therapies and immunotherapies, the only treatment for patients with advanced melanoma was dacarbazine. However, as this rather toxic chemotherapy did not provide any survival benefit, a diminished ability to tolerate intensive chemotherapy does not explain the survival discrepancy between the young and old (1).

Notably, older patients tend to present with thicker primary tumors (Breslow > 4 mm, a definition of high-risk primary disease) more often than younger patients (1), and despite being localized to the skin at diagnosis, the overall survival for patients with thick melanoma is only 45% to 60%, with a gradual decline in survival with increasing decades of life. Multivariate analyses demonstrate that increasing age is the strongest independent adverse prognostic factor, together with Breslow thickness (2). Other markers of poor prognosis, such as ulceration and elevated mitotic count in the primary tumor, are also more common in the elderly, but, paradoxically, elderly patients are less likely to have lymph node metastases than younger patients (3). The lower likelihood of regional nodal metastasis in patients who are at higher risk of distant recurrence suggests a different biology of metastatic progression. Recently, the recruitment of neutrophils to the primary melanoma in mice was found to drive the perivascular invasion of melanoma cells, likely increasing the risk for hematogenous spread (4). The substantial neutrophilic infiltrate present in ulcerated primary melanomas, which are more common in elderly patients, affords a plausible explanation for a worse outcome and a more frequent hematogenous pattern of metastasis in this patient population.

Now, in an elegant recent study, Kaur and colleagues (5) have unraveled an intriguing role for age-related alterations in dermal fibroblasts that drive melanoma progression and response to therapy. To study whether an aged microenvironment favors melanoma progression, mouse-derived BrafV600E/Cdkn2a−/−/Pten−/− melanoma cells were injected subcutaneously into young mice and into mice reaching the end of their lifespan. Intriguingly, the tumors established in older animals grew more slowly, but presented higher blood vessel density and developed more lung micrometastases. Using an organotypic model, they demonstrated that melanoma in skin reconstructs built with aged fibroblasts invaded more efficiently and presented a lower proliferation rate than in skin reconstructs built with fibroblasts from young donors. In vitro, they examined the conditioned media from fibroblast cultures and found that sFRP2 secretion was significantly higher in aged fibroblasts. They tested the role of this factor in vitro by driving recombinant sFRP2 expression in young fibroblasts or silencing sFRP2 in aged fibroblasts to produce consistent results, and in vivo they showed that in young mice increased serum sFRP2 increased tumor blood vessel density and lung metastasis.

Critically, sFRP2 inhibits β-catenin, which is known to promote melanoma proliferation, while inhibiting its invasion. They demonstrated that β-catenin expression was decreased in human aged skin and in melanoma cells treated with recombinant sFRP2 or conditioned medium from aged fibroblasts. Importantly, knockdown of β-catenin in young fibroblasts increased sFRP2 secretion and drove invasion of melanoma cells expressing low β-catenin. Moreover, MITF, the master regulator of melanocyte function, is activated by β-catenin, and when β-catenin was decreased, there was dysregulation in the redox pathway downstream of MITF due to reduced expression of the redox effector APE1, dampening the response to reactive oxygen species (ROS) and increasing oxidative damage in aged skin. Importantly, the impaired response to oxidative stress rendered melanoma cells more vulnerable to DNA damage and, accordingly, stimulated robust expression of DNA damage markers (γH2AX and 53BP1) in melanoma cells from aged mice following exposure to conditioned media from aged fibroblasts, and in skin reconstructs built with aged fibroblasts. This damage was reversed by an antioxidant that decreased ROS production, with the intriguing implication that age-mediated damage could be treated by reducing ROS. Pleasingly, they validated their findings by showing that elderly patients with melanoma had high levels...
Figure 1. Aged fibroblasts drive melanoma cell metastasis and resistance to BRAF inhibitors (BRAFi). As fibroblasts in the skin grow older, they upregulate secretion of the WNT antagonist sFRP2. sFRP2 antagonizes β-catenin and MITF signaling in melanoma cells, suppressing APE1 expression and reducing the cells’ ability to combat ROS-induced damage, thereby driving resistance to BRAF inhibitors.

of sFRP2 in serum, fibroblasts, and melanoma cells, and that this was accompanied by reduced β-catenin, MITF, and APE1 expression, and increased evidence of oxidative damage in melanoma cells.

Because elevated ROS expression, reduced β-catenin, and MITF have all been linked to resistance to targeted therapy with BRAF inhibitors, the authors tested the role of aged fibroblasts as possible drivers of resistance. They found that melanoma spheroids exposed to young fibroblast media were more sensitive to the BRAF inhibitor PLX4720 than those exposed to aged media (Fig. 1). Moreover, tumors in aged mice were less responsive to PLX4720. This observation was validated by pretreating melanoma cells exposed to young media with hydrogen peroxide, which led to BRAF inhibitor resistance. In contrast, aged fibroblast media pretreated with a powerful antioxidant increased melanoma cell death, and, because β-catenin loss contributes to resistance, they administered recombinant sFRP2 to tumor-bearing young mice and showed that their tumors rapidly became resistant to PLX4720. In humans, they showed that patients under 65 presented significantly more tumor burden reduction compared with patients older than 65.

The data presented in this study highlight the relevance of the microenvironment as a modulator of melanoma progression and therapy response, and the mechanistic insights provided are relevant to the aged microenvironment and patient. More importantly, the findings revealed biological pathways in melanoma progression and therapy response relevant to all patients. One of the more intriguing findings reported here is that melanoma cell death was induced by exposure to antioxidant-treated aged fibroblast media. This striking result is especially significant because highly glycolytic KRAS- or BRAF-mutated colorectal cancer cells are vulnerable to treatment with oxidized vitamin C, which upon reduction generates ROS, depletion of glutathione, and an energetic cellular crisis that limits tumor growth in mice (6). These remarkable findings are in sharp contrast to the reported detrimental effect in melanoma cells, where antioxidants have been shown to promote tumor initiation (7), melanoma cell invasion in vitro (8), and metastasis in mouse models (9). It is entirely plausible that different cell types process antioxidants differently, and further studies are needed to understand whether the protective effect observed in this study is limited to melanoma in the elderly.

Importantly, there is an unstudied range of stromal and fibroblast density observed across different melanoma subtypes, affording varying degrees of heterocellularity that might provide unique microenvironments that affect melanoma biology differently in particular circumstances. It is intriguing that fibroblast depletion in epithelial pancreatic cancer in mice leads to accelerated death and alters the immune cell infiltrate in primary tumors (10). Because immune cells infiltrating primary melanoma are found to be critical predictors of immunotherapy response, it will be interesting to see how these findings affect tumor immune cell infiltrates in the elderly and the young. The model presented provides a new tool to study biology and therapies in the specific population that is most affected by melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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