The Ups and Downs of Transcription Factors in Melanoma

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Of the three key developmental pathways, NOTCH, WNT, and Sonic Hedgehog (SHH), the least is known about the SHH pathway in melanocyte development and melanoma. In this issue of the Journal, a study from the laboratory of Alain Mauviel is the first to connect transforming growth factor-β (TGF-β) with SHH signaling in promoting melanoma metastasis. Alexaki et al. (1) reveal that GLI2, a transcription factor traditionally downstream of SHH activation, is overexpressed in human melanoma. Melanoma cells in which GLI2 expression was higher than normal (GLI2-high cells) metastasized to bone more readily than cells in which GLI2 expression was lower (GLI2-low cells), and reduction of GLI2 expression impaired bone metastasis (1). It is known that TGF-β overexpression stimulates increased matrix protein secretion, adhesion receptor expression, and invasion of melanoma cells (2), and previous work from this group revealed GLI2 as a direct target of TGF-β and SMAD signaling (3). The current work clearly demonstrates that activation of GLI2 promotes invasion and metastasis and suppresses E-cadherin expression leading to a mesenchymal-like phenotype. Thus, TGF-β regulates invasion and migration through a novel mechanism utilizing a SHH pathway component. Likely because of deregulation of this stimulatory signaling mechanism, tumor cells often become SHH independent, and endogenous or exogenous SHH has no effect on melanoma growth and migration (M. Fukunaga-Kalabis and M. Herlyn, unpublished data). SHH antagonists, such as cyclopamine, have little effect on melanoma cells in vitro, suggesting that TGF-β hijacks the transcriptional machinery of the SHH pathway (M. Fukunaga-Kalabis and M. Herlyn, unpublished data). Stecca et al. (4) previously reported that GLI1, a direct GLI2 target, is required for normal melanocyte proliferation as well as growth and metastasis of melanoma cells. Therefore, GLI2 is emerging as a common target of the developmental machinery and a progression factor in melanoma.

Remaining questions include how the other developmental pathways are activated in melanoma. The NOTCH pathway is constitutively active in melanomas, and expression of activated NOTCH1 induces a transformed phenotype in melanocytes (5). However, the mechanism for constitutive NOTCH activation in metastatic melanoma is unknown. Meanwhile, canonical WNT signaling is essential for melanocyte differentiation. During melanoma progression, a switch from canonical (WNT1 and WNT3A) to noncanonical (WNT5A) Wnt signaling occurs (6). Similar to SHH, these pathways are also hijacked, but likely more upstream, at the receptor rather than the transcriptional level.

Also in this issue, a report from the laboratory of Gavin Santiago-Walker highlights the importance of the tumor microenvironment and cell–matrix signaling in tumor progression. The article by Huh et al. (7) is the first to functionally describe KLF6 as the proposed second tumor suppressor gene on chromosome 10 in melanoma. In addition to the previously described loss of PTEN at 10q23 (8–10), Huh et al. show that loss of the KLF6 gene at 10p15 results in an increase in proliferation and survival of melanoma cell lines, but interestingly, only in the presence of collagen and not when cells were maintained on plastic. Overexpression of KLF6 in human melanoma cells restored the inhibitory effect of collagen on melanoma cell proliferation. As expected, KLF6 was decreased in aggressive human melanomas in vivo, again suggesting that KLF6 mediates inhibitory signals from the matrix environment. There are several examples in the literature in which changes in gene expression are only observed when cells were maintained in collagen. For example, biologically early melanoma cells that overexpressed the vitronectin receptor αvβ3 became highly invasive if cells were grown in three-dimensional cultures in the presence of collagen, whereas no change was observed in cultures grown on plastic (11). Similarly, cells overexpressing the melanoma antigen MCAM exhibited increased invasiveness in collagen but not when maintained in conventional culture (12). These data support the conclusion of the Huh report that describes KLF6 as a collagen type-1-dependent tumor suppressor in melanoma.

Another KLF family member, KLF4, has gained prominence for its ability to reprogram fibroblasts, keratinocytes, and melanocytes to embryonic stem cell–like cells (13). KLF6, which is involved in hematopoiesis and adipocyte differentiation (14), could potentially promote melanocyte differentiation, rather than the dedifferentiation induced by its family member KLF4. Melanomas express the collagen receptors α1, α2, and α3 and often produce several collagens. Thus, melanomas must eliminate KLF6 if collagen receptors are engaged. Possibly, loss of KLF6 is an escape mechanism from the ever-present inhibitory collagen signals in the melanoma tumor environment. Therefore, melanoma cells are under selective pressure to lose KLF6 if they are to progress to the tumor-forming stage.

The discovery of KLF6 as a conditional matrix-dependent tumor suppressor gene sparks our interest in its role in melanocyte regulation. Work from our laboratory has shown a role for the matricellular protein CCN3 (nephroblastoma overexpressed) in melanocyte homeostasis. CCN3, by inducing the receptor tyrosine kinase discoidin domain receptor 1 (DDR1), promotes adhesion to collagen IV for melanocyte proliferation. It would be interesting to determine what involvement, if any, KLF6 has in mediating...
signals from collagen IV to CCN3 and/or DDR in melanocyte homeostasis.

The link between the two excellent studies from the Mauviel and Robertson laboratories is the matrix. TGF-β is the dominant growth factor for matrix induction and adhesion receptor expression, whereas loss of KLF6 expression allows escape from collagen-mediated growth inhibition. Clearly, the tumor microenvironment cannot be ignored if we are to understand the ups and downs of melanoma signaling leading to disease progression.

References


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