Aiolos and Lymphocyte Mimicry in Lung Cancer

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Aggressive carcinomas tend to adopt behaviors normally restricted to lymphocytes, including anchorage-independent mobilization, response to chemokines, and modulation of local inflammatory conditions. In a recent study we identified the lymphocyte-restricted chromatin regulator Aiolos as an epigenetic driver of lymphocyte mimicry in lung cancer that links immune cell development to metastatic behavior.

Blood cells require fewer mutations than epithelial cells to become malignant, in part due to their intrinsic anchorage independence and ability to traffic throughout the body. Epithelial cells, by contrast, have strong anchorage requirements that must be separately overcome during transformation, resulting in the acquisition of hematopoietic cell properties. However, the factors responsible for reassigning epithelial cell identity during tumor evolution have been elusive.

Aiolos and its close relative Ikaros are transcription factors that are primarily restricted to hematopoietic cells. In seminal work, Ikaros and Aiolos were shown to be master cell fate determinants driving overlapping stages of lymphopoiesis.1,2 Both proteins appear to specify lymphocyte lineage by recruiting chromatin modifiers to a number of loci, causing gene silencing or activation through chromatin regulation. Of interest, expression of Aiolos has been observed in several solid tumors and cell lines, raising the possibility that aberrant expression of this protein may redirect cell lineage in epigenetically unstable carcinomas, leading to a metastatic phenotype.

We explored this hypothesis in a recent publication focusing on the expression of Aiolos (encoded by IKZF3) in lung cancer cells.3 In immunohistochemical studies, we found that the majority of non-small cell lung cancer (NSCLC) and all small cell lung cancer (SCLC) tumors expressed detectable levels of Aiolos protein. When staining intensity was scored, roughly half (46%) of advanced-stage NSCLC tumors expressed high levels of Aiolos. Of note, a similar proportion of early-stage tumors were scored as high Aiolos expressers (53%), suggesting that IKZF3 induction frequently occurs prior to clinical detection of lung cancers. When survival rates of NSCLC were analyzed, high Aiolos expression predicted a markedly worse outcome. Interestingly, given the poor prognosis and lymphoma-like behavior of SCLC, all SCLC tumors examined expressed high levels of Aiolos.

Gene expression profiling and functional studies in A549 lung cancer cells demonstrated that a major effect of IKZF3 expression is deregulation of cell adhesion. The most highly enriched functional classes of repressed genes belonged to cellular adhesion categories, with downregulation of more than 80 genes controlling cell matrix, focal adhesion, cytoskeletal, and cell–cell junctional proteins. Functional studies confirmed that Aiolos causes rounding of lung cancer cells with decreased matrix adhesion and loss of intercellular junctions. Aiolos also confers prominent anchorage independence in vitro and markedly increases the metastatic behavior of lung cancer cells in mice.

A key Aiolos target gene with respect to controlling cell behavior is SHCI, which provides an anchorage context to cell fate signaling. SHCI contains tandem promoters that independently regulate expression of two proteins with opposite function: p52Shc is ubiquitously expressed and pro-mitogenic, whereas p66Shc enforces anchorage dependence and anoikis, functions as a powerful metastasis suppressor in mice, and is repressed in normal hematopoietic cells and metastatic SCLC cells.2 We identified a critical p66Shc-specific enhancer and found that expression of p66Shc in normal epithelioid cells requires complex higher-order chromatin organization that involves tethering of the enhancer and three other cis-acting regions to their cognate promoter. In lung cancer cells, Aiolos binds multiple sites of p66Shc including the enhancer to reconfigure chromatin structure, deacetylate promoter histones, and selectively silence gene expression. Thus Aiolos strongly promotes anchorage independence through epigenetic control of SHCI and other genes (Fig. 1).

Clues regarding the biological significance of these findings can be found by considering normal lymphocyte development. Aiolos expression is first detected in lymphoid progenitors and increases throughout maturation of T and B cells,2 a stage of lymphocyte development that coincides with downregulation of adhesion-related genes and sustained loss of lymphocyte adhesion to matrix-rich bone marrow and thymus niches. The shift in expression pattern suggests that Aiolos may direct epigenome-wide changes that facilitate the departure of lymphocytes or in the case of lung cancer, epithelial cells, from their respective matrix-rich developmental niches to permit survival in the circulation. Importantly, a recent report demonstrated that deletion of Ikaros (encoding Ikaros) in mouse lymphoid progenitors increases expression of multiple genes controlling matrix adhesion and...

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signaling, and causes the cells to flatten and assume an epithelioid morphology.\textsuperscript{3} \textit{Ikaros} null lymphoid progenitors became anchorage dependent, displaying anoikis upon loss of stromal contact. These changes are essentially the opposite of those observed upon expression of \textit{IKZF3} in human lung cancers, consistent with the interpretation that Aiolos (and perhaps also Ikaros) activates normal lymphoid developmental programs in epithelial carcinomas.

Interestingly, we found that Aiolos also induces lymphocyte genes such as CD22 and CXCR4, which encode homing receptors, in lung cancer cells. CXCR4 normally guides hematopoietic cells to immunologically active sites such as bone marrow, lymph nodes, lung, and liver – sites that are frequently colonized by metastatic carcinomas. Recognition that breast cancer cells express CXCR4 was perhaps the first molecular clue that solid tumors with poor prognosis engage in hematopoietic cell mimicry;\textsuperscript{4} our study suggests that chemokine receptor expression may be part of a larger epigenetic reprogramming event driven by Aiolos. The full extent of such lymphocyte-like behavior has yet to be fully explored. For example, Aiolos is known to be upregulated during the commitment of naïve T cells to immunosuppressive regulatory T and inflammatory \textit{T}_{\text{H}17} subsets,\textsuperscript{5,6,7} both of which are implicated in tumorigenesis. Whether Aiolos enables solid tumors to modulate their immune microenvironment will be an important area for further research. Of note, thalidomide and its analog lenalidomide, two prototype immune modulatory drugs, were recently found to exert their antitumor effects against multiple myeloma through selective protosomal degradation of Aiolos and Ikaros,\textsuperscript{8,9,10} suggesting that these chromatin regulators may control aspects of tumor immunity.

Equivalent to genetic driver mutations, Aiolos appears to be a key epigenetic driver capable of partially reassigning lineage commitment in lung cancer, and possibly other solid cancers. Our study suggests that Aiolos may cause heritable changes in cell identity that endow epithelial cancers with lymphocyte properties, in essence causing an incomplete epithelial-to-immune cell transition. Of note, such lineage plasticity through extensive chromatin remodeling is inherent in lymphocyte development itself. Further insights into the nature of such a transition might provide novel targets for future intervention.

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