Pharmacologic Blockade of a Pioneer Transcription Factor

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Cancers frequently co-opt lineage-specific transcription factors (TF) utilized in normal development to sustain proliferation. However, the effects of these TFs on tumor development depend considerably on where in the genome they bind. A new article by Taylor and colleagues expands on previously developed diamidine compounds that obstruct the DNA binding sites of the pioneer TF PU.1 (SPI1) in acute myeloid leukemia. Immobilization and sequencing of genomic DNA targeted by these compounds revealed that these inhibitors alter the genomic binding patterns of PU.1. The authors report that their strategy constrains the genomic binding preferences of PU.1, leading to redistribution of PU.1 to promoters and other gene-proximal regions with elevated guanine/cytosine content. In this study, we discuss recent developments for targeting PU.1 in hematologic malignancies. We also explore the shared functional roles of PU.1 and SWI/SNF ATP-dependent chromatin remodeling complexes, which not only work together to sustain the enhancer landscape needed for tumor cell proliferation but also play key roles in nontumor settings.

In several malignancies, a small number of transcription factors (TF) are recurrently hijacked to sustain tumor growth. Across diverse cancer types, the TFs with oncogenic activities show a striking overlap with TFs that also support nontumor progenitor cells from the same lineage. For example, acute myeloid leukemia (AML) strongly depends on TFs involved in hematologic development, whereas tumors of the epithelial, nervous, and endocrine systems each exhibit distinct TF dependencies strongly linked to their lineage of origin. Across many cancer types, disruption of tumor TF circuitries impairs G_1–S transition, culminating in differentiation and/or loss of tumor cell viability (1). Hence, cancers hijack and corrupt lineage-specific TF circuitries to enable constitutive replication commitment in the absence of growth signals.

Among these diverse cancer contexts, AML has emerged as an ideal setting for mechanistic dissection and pharmacologic targeting of tumor-essential TFs. As reported last year in *Cancer Research*, the ETS family member known as PU.1 (SPI1) plays an early role in the establishment of key enhancers in AML (2). As a pioneer TF, PU.1 has the capacity to bind poorly accessible regions and associates with chromatin largely independently of the ATPase activity of SWI/SNF ATP-dependent remodeling complexes. Nevertheless, PU.1 directly binds (3, 4) and recruits SWI/SNF complexes to its sites (2). The local increase in DNA accessibility following SWI/SNF remodeling at PU.1 sites enables binding of downstream TFs, such as RUNX1, MEIS1, or LMO2, which work together at enhancers to drive the expression of MYC and other targets (2).

Although therapeutic targeting of TFs is often challenging, several approaches to indirectly interfere with PU.1 activity have had considerable success (**Fig. 1**). The recent development of selective SWI/SNF ATPase inhibitors provides an avenue to impair PU.1 pioneer–directed enhancers in AML. Although SWI/SNF inhibition preserves PU.1 binding at the majority of its sites across the genome, these sites lose accessibility and the occupancy of downstream TFs that depend on SWI/SNF-generated accessibility (2). Additionally, this strategy also leads to accumulation of PU.1 at promoters involved in myeloid differentiation, and hence chemical inhibition of SWI/SNF leads to redistribution of PU.1 across the genome. In murine models of AML, SWI/SNF inhibition causes rapid leukemic regression and is well tolerated. Additionally, primary human AML samples are responsive at nanomolar concentrations of SWI/SNF inhibitors.

Another approach to disrupting PU.1-directed enhancers is the use of heterocyclic diamidine compounds that bind to DNA and interfere with PU.1's ability to bind its target sites. Diamidine inhibitors selectively impair PU.1 binding to its target sites on DNA (5) and thereby also prevent SWI/SNF recruitment (2). Treatment with these compounds leads to reduced DNA accessibility at PU.1 binding sites and causes similar genome-wide expression patterns as SWI/SNF inhibition. Impairment of either individual step is sufficient to induce MYC loss and to cause myeloid differentiation, reflecting a key shared function of the PU.1–SWI/SNF regulatory axis in AML. The resulting myeloid differentiation observed in both cases is a potentially beneficial therapeutic outcome that moreover underscores the strong functional stepwise linkage between pioneer TF binding and chromatin remodeling.

In a recent publication (6), Taylor and colleagues build on their earlier development of diamidine compounds that impair PU.1 binding and introduce an approach they term "CLICK-on-CUT&Tag." In this system, the diamidine DB2750 is covalently coupled to biotin and immobilized on streptavidin beads. The ability of DB2750-coated beads to bind the DNA from PU.1 CUT&Tag enables the identification of the specific genome-wide sites that are targeted by PU.1 and also bound by DB2750, thereby revealing the genome-wide functional targets of the compound.

By using DB2750 and variants in cell lines, Taylor and colleagues uncover that these compounds induce repositioning rather than global loss of genomic PU.1 sites. By analyzing the sequence specificity of affected sites, they report that long-term treatment with DB2750 induces guanine/cytosine (G/C) sequence bias immediately flanking the PU.1 consensus motif at regions associated with differential increases in PU.1 binding. Primary AML samples respond with myeloid differentiation and loss of colony formation potential at concentrations between 1 and 5 μmol/L, suggesting that further development of diamidine compounds could also be therapeutically beneficial. Similar to previous observations with SWI/SNF inhibition (2), the authors observed that

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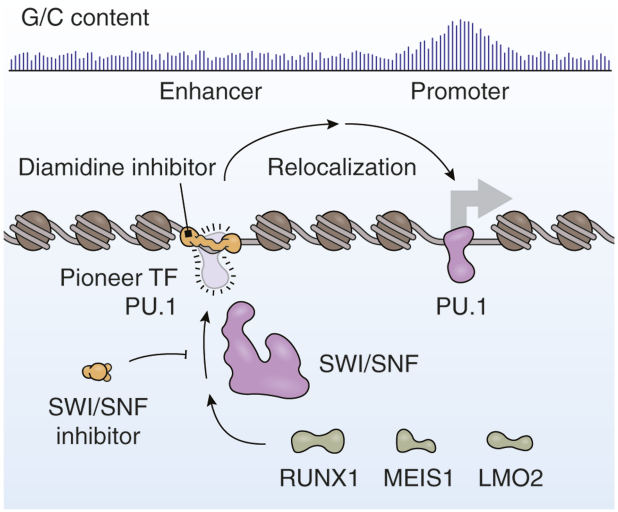

Figure 1.

Strategies for pharmacologic targeting of the pioneer TF PU.1. Diamidine compounds obstruct availability of PU.1 DNA binding sites, and SWI/SNF inhibition limits the generation of DNA accessibility, which prevents downstream TFs from binding. Both processes result in redistribution of PU.1 binding sites across the genome, a process that coincides with myeloid differentiation in AML.

PU.1 redistribution results in increased PU.1 occupancy at promoters, the majority of which occurs at a later time, coincident with myeloid differentiation.

The ability to chemically target and perhaps directly redistribute a pioneer TF raises many exciting questions that will undoubtedly be addressed in the future. Promoters are naturally enriched in G/C nucleotide content, and the resulting accumulation of PU.1 at these sites observed in cell lines is much slower than their losses elsewhere in the genome. This finding raises the question of whether the observed sequence bias of PU.1 redistribution reflects a direct, sequence-specific effect of the diamidine compounds, or instead arises as an indirect consequence of other biological features, such as chromatin marks, differences in the regulatory machinery at promoters and distal enhancers, or other differentiation-specific activities that may stabilize PU.1 at gene-proximal regions. Additionally, it remains unclear whether TF redistributors such as DB2750 are reversible or induce permanent relocalization because of differentiation. The previous observation that SWI/SNF inhibition leads to similar accumulation of PU.1 at myeloid-specific promoters associated with differentiation also raises the question of whether accumulation of PU.1 at promoters drives cell differentiation or reflects it.

In addition to AML, many hematologic cell types use the PU.1–SWI/SNF regulatory axis for both cancer-specific as well as normal functions. For example, SWI/SNF inhibition induces modest leukopenia *in vivo*, with reduced production of B cells and monocytes (2), two lineages that require PU.1 during their development. In this setting, a similar accumulation of DNA accessibility at PU.1 sites at promoters is observed in circulating B cells and monocytes, suggesting that PU.1 redistribution also influences nontumor cell types. Indeed, PU.1 and SWI/SNF similarly cooperate to enable NF-κB enhancer binding in both lymphomas and prememory B cells (7). Despite increasing evidence that PU.1 and SWI/SNF play important roles in T cells (8, 9), Taylor and colleagues observe more modest effects on the Jurkat T lymphoblast cell line, and hence many questions remain about the cell type–specific functions of this regulatory axis.

Therapeutic approaches targeting TFs or their coactivators can also impact normal cells, as these cells often use the same TFs that tumor cells use to drive proliferation. As a result, a viable therapeutic window for such approaches requires that tumor cells have greater need for these activities than other essential cell types. Indeed, targeting the PU.1–SWI/SNF regulatory axis in MLL–AF9 (2) and FLT3–ITD (10) murine models of AML causes disproportionate reduction of leukemic stem cells, suggesting an unusually heightened dependency on this regulatory axis in certain leukemic stem cells. If the concept of selective TF redistributors can be rationally extended to other TFs, such an approach could be envisioned to enable powerful opportunities to precisely target specific cell types by targeting multiple TFs. In such a scenario, the combined inhibition of multiple TFs might provide new avenues to target tumor-specific circuitries from diverse lineages with high potency and specificity.

Authors' Disclosures

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