Peptide inhibitor of methylation casts new light on epigenetic-based cancer therapy

Review Article

The epigenome plays a key role in gene expression and epigenetic changes are thought to be involved in many, perhaps all cancers, which makes methylation modulators promising drug candidates. The chromatin modulator DPY30 facilitates histone H3K4 methylation by directly binding to ASH2L in the SET1/*MLL* complexes and plays an important role in hematologic malignancies. A team based at the Department of Biochemistry and Molecular Genetics, University of Alabama, USA, developed a peptide to disrupt binding of DPY30 to ASH2L of the SET1/*MLL* complex to investigate DPY30's activity in cancer growth and explore the potential of targeting this chromatin modulator to inhibit cancer.

Epigenetics puts a new layer on cancer therapy

Epigenetic changes can be reversed, which makes them attractive targets for therapy, and clinical trials are in progress to examine the potential of several small molecules that target chromatin-based mechanisms (1). To date, the FDA has approved eleven drugs with epigenetic effects. Seven target different kinds of cancer by upregulating tumor suppressor genes. These drugs all target hematopoietic cancers, which are more sensitive to these types of drugs.

The first peptide-derived epigenetic drug (Romidepsin) was approved in 2009 for the treatment of cutaneous T cell lymphomas (CTCL), and in 2011 for peripheral T cell lymphomas (PTCL) (2). Romidepsin also has potential in the treatment of other kinds of cancer, lung fibrosis, and Epstein-Barr infections. Currently, it is the only FDA-approved peptide medicine specifically designed to target epigenetic effects. Nesiritide, a peptide originally approved in 2001 for the symptomatic treatment of acute decompensated heart failure due to its vasodilating activity has since been repurposed due to its epigenetic effects. These peptides are not yet approved in the European Union by the EMA.

SET1/*MLL* and DPY30 in tumorigenesis

The SET1/*MLL* complexes are major methylation enzymes of histone H3K4 in mammals. These complexes comprise a number of subunits that are important for efficient H3K4 methylation, including DPY30, which has roles in regulating fundamental cell processes including growth, differentiation, and senescence, especially in the hematopoietic system, and is involved in tumorigenesis. For example, genes encoding DPY30 and other SET1/*MLL* subunits are amplified in a wide range of human cancers, DPY30 depletion reduces c-MYC (MYC) expression and growth of several *MLL*-rearranged leukemias. DPY30 is also significantly upregulated in primary MYC-translocated Burkitt's lymphoma. DPY30 therefore plays a key role in regulating MYC-dependent lymphomagenesis, making it a potential drug target in the treatment of lymphomas.

Targeting DPY30 with peptides

DPY30 enhances SET1/*MLL* complex methylation activity by binding through its C-terminal domain (residues 45–99) to a 14-residue C-terminal region of the ASH2L subunit. Binding involves dimerization (or weak oligomerization) of the DPY30 C-terminal to form a semi-circular hydrophobic groove that accommodates the amphipathic α-helix of the ASH2L C-terminal region. While this interaction is key for enhancing H3K4 methylation, it has not been shown to be important in regulating tumorigenesis. The team based at the University of Alabama therefore designed cell-penetrating peptides to disrupt the interaction between DPY30 and ASH2L to investigate its anti-tumor effects (3).

Peptide design and synthesis

Three peptides were designed based on a peptide-interference strategy shown to inhibit B-cell lymphomas. These included a peptide comprising the HIV TAT protein-transduction motif to ensure cell penetration, an HA epitope tag, and the ASH2L (510–529) sequence (WT, Fig. 1). A control peptide was designed that included mutations of three key hydrophobic residues of ASH2L known to disrupt binding to DPY30 (3R, Fig. 1). An extra control peptide was designed to study specific effects, such as the potential cellular effects of increased DPY30 binding and include a point mutation of tyrosine 518 to arginine (Y518R, Fig. 1) that boosted ASH2L binding to DPY30 10-fold.

Figure 1. The three peptides tested, showing the TAT, HA and ASH2L regions. From Figure 1A, Shah et al, 2019.

The peptides were synthesized using a standard, double-coupling, FMOC, solid-phase peptide synthesis strategy on Prelude®. Following deprotection and cleavage from the resin, peptides were purified by HPLC and confirmed by mass spectrometry to be over 95% purity. The peptides were lyophilized and stored at -20°C until reconstituted with sterile and deionized water. Peptide WT was FITC-labeled to perform a fluorescence polarization assay (FPA).

WT peptide disrupts DPY30/ASH2L binding and inhibits *MLL*-based cancer cell line growth

A series of experiments showed that the peptide WT specifically disrupts the binding of DPY30 to ASH2L and inhibits the growth of leukemia cell lines driven by *MLL* rearrangements (Table 1). These results indicated that the DPY30-binding peptides specifically inhibit leukemia cell growth and clonogenicity.

Table 1: Characterization of peptide binding and inhibitory properties

	Peptide		
Characteristic	**WT**	**3R control**	**Y518R**
Cell entry and transport to nucleus	Yes	Yes	Yes
Binding to DPY30 (pull-down expt.)	Yes	No	
Binding to ASH2L (FPA; IC_{50})	2 µM	>>2 µM	1 µM
Inhibition of DPY30 binding to ASH2L	Yes	No	
Inhibition of DPY30-stimulation of methylation	Yes	No	Yes
Inhibition of cell growth of leukemia cell lines driven by *MLL* rearrangements	Yes	No	Yes
Inhibition of growth of leukemia cell line driven by non-*MLL* rearrangements	No	No	No
Inhibition of normal human $CD34^+$ cells	No	No	No
Inhibition of colony formation of MOLM-13 cells (an *MLL*-AF9-driven leukemia cell line)	Yes	No	Yes
Inhibition of MYC-dependent blood cancer cell lines (Burkitt's lymphoma, acute T cell leukemia)	Yes	No	Yes

DPY30-binding peptides affect gene expression

RNA sequencing of *MLL*-rearranged leukemia cell lines, MYC-dependent blood cancer cells, and a K562 cell control exposed to Y518R or 3R control peptide showed a large difference in response between the cancer cell lines and the control (Fig. 2). These results explain the molecular basis for the selectivity of the DPY30-binding peptides for these types of blood cancers against the K562 leukemia cells.

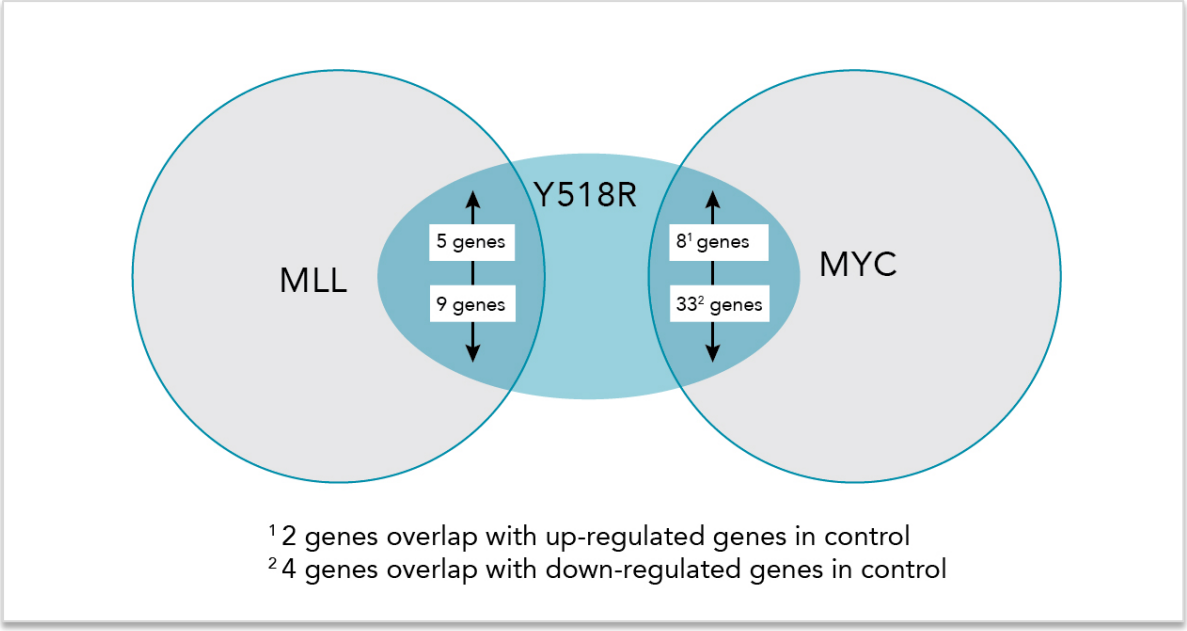

Figure 2. A summary of the up- and down-regulation of genes in *MLL*-rearranged leukemia cell lines (*MLL*), and MYC-dependent blood cancer cells (MYC) by the DPY30-binding peptide Y518R.

DPY30-inhibitory peptide increases sensitivity to other epigenetic inhibitors

The extensive cross talk among epigenetic modifications opens up the possibility of combining DPY30-binding peptides with existing epigenetic intervention methods for cancer treatment. Tests on MOLM-13 cells showed that Y518R sensitizes the *MLL*-rearranged leukemia to BET family inhibitors, and also EZH2 inhibitors.

A promising epigenetic approach to fight hematologic malignancy

These results show the potential for peptides targeting DPY30/ASHL2 binding as a method of treating hematologic malignancy. The next step could be the development of even more effective molecules to target DPY30, based on peptidomimetics and small molecule inhibitors with better pharmacological properties.

References

1. Exploiting the epigenome to control cancer promoting gene expression programs. Brien, GL *et al*. Cancer Cell. 2016 April 11; 29(4): 464–476. doi:10.1016/j.ccell.2016.03.007.
2. Peptides as epigenetic modulators: therapeutic implications. Janssens, Y et al. Clinical Epigenetics (2019) 11:101. https://www.ncbi.nlm.nih.gov/pubmed/31300053
3. Specific inhibition of DPY30 activity by ASH2L-derived peptides suppresses blood cancer cell growth. Shah, KK *et al*. Experimental Cell Research. Vol 382, Issue 2, 15 September 2019, 111485.