

NAD as a Genotype-Specific Drug Target

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Using high-throughput chemical and genetic screening, Matheny and colleagues (in this issue of *Chemistry & Biology*) identified STF-118804, an inhibitor of nicotinamide phosphoribosyltransferase, as a cell type-specific inhibitor of mixed-lineage leukemia with *MLL* chromosomal rearrangements. The approach was powerful, as is the potential for NAD as a specific cancer target.

Philadelphia chromosome-positive chronic myelogenous leukemia remains the paragon of genotype-targeted cancer pharmacology. The identification of ABL kinase activation by *BCR-ABL* fusion and the availability of STI-571 as an ABL-targeted inhibitor have allowed hundreds of thousands of US patients to be treated with a specific agent (Druker, 2008). Childhood acute lymphoblastic leukemias (ALL) that are refractory to treatment frequently contain chromosome translocations of the mixed lineage leukemia (*MLL*) gene (de Boer et al., 2013; Muntean and Hess, 2012). The *MLL* gene encodes a histone H3 Lys 4 methyltransferase that is essential for *HOX* gene expression during development. Although mixed lineage leukemia (*MLL*) translocations result in loss of the endogenous methyltransferase domain, oncogenic *MLL* fusion proteins are associated with a set of transcriptional elongation promoting proteins and the histone H3 Lys79 methyltransferase DOT1L, which further enhance target gene expression (de Boer et al., 2013; Muntean and Hess, 2012). In addition, the N-terminal menin-binding motif of *MLL* and *MLL* fusions is essential for interactions with menin, the lens epithelium-derived growth factor, and chromatin and is required for leukemogenesis (Muntean and Hess, 2012). Thus, multiple *MLL*-associated factors are potential drug targets for *MLL*-rearranged ALL (de Boer et al., 2013; Muntean and Hess, 2012).

Motivated by the severity of the malignancy and the wealth of potential drug targets, Matheny et al. (2013; in this issue of *Chemistry & Biology*) conducted a cellular high-throughput screen for lead compounds that would inhibit proliferation of *MLL*-rearranged ALL and possess little activity against ALL cell lines without

MLL gene rearrangement. STF-118804 was identified in such screens. To identify the molecular target of this compound, they infected an *MLL*-rearranged cell line with pools of an ~9,300 gene-targeting shRNA library, which contains ~25 knockdown reagents per gene and identified the shRNAs that were depleted by selection with STF-118804. The data indicated that silencing of the nicotinamide phosphoribosyltransferase gene (*NAMPT*) produced hypersensitivity to the chemical probe (Matheny et al., 2013).

NAMPT is the enzyme required for salvage biosynthesis from nicotinamide to nicotinamide adenine dinucleotide (NAD). Nicotinamide, like the other NAD precursor vitamins nicotinate and nicotinamide riboside, is a water soluble 3-pyridyl-containing metabolite. STF-118804 bears a strong resemblance to *Nampt* inhibitors FK866 and CB30865. All three are 3-pyridyl compounds with a hydrophobic extension (Figure 1). NAD and its reduced and phosphorylated forms, NADH, NADP, and NADPH, are essential for the function of multiple oxidoreductases in central carbon metabolism. This is termed the redox function of NAD. In addition, NAD is a consumed substrate of Sir2-related protein Lys deacetylases (sirtuins) and ADP-ribose-transfer enzymes including poly(ADP-ribose) polymerase (PARP). Nonredox functions of NAD are mediated by enzymes, such as sirtuins and PARP, that degrade the NAD dinucleotide to nicotinamide and ADP-ribose products, necessitating nicotinamide salvage in order to maintain NAD homeostasis (Belenky et al., 2007).

NADH is so central to life that many common cell viability assays measure it as a proxy for proliferation. Cellular reduction of tetrazolium salts such as 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-

tetrazolium bromide, and phenoxazine dyes such as resazurin (sold as Cell Titer Blue) are commonly employed in microtiter assays of proliferation and viability. Because these assays principally report on NADH-dependent reduction rather than cell viability, they are ideally suited to identify NAD-targeting compounds. Thus, the identification of a *Nampt* inhibitor as antiproliferative could have been a trivial result, because inhibition of NAD salvage necessarily limits the amount of NADH available to reduce resazurin independent of whether cells are viable. However, the investigators clearly showed that *MLL*-rearranged cell lines are hypersensitive to STF-118804-induced apoptosis (Matheny et al., 2013). Why then are rearranged *MLL* cells so sensitive to targeting NAD?

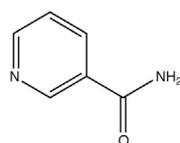
Just as NAD is involved in redox biology and non-redox biology, mechanisms for genotype-specific cytotoxicity fall into two classes. *MLL* fusion complexes play established roles in epigenetic programming. If *MLL*-rearranged cells induce the activity of NAD-consuming enzymes such as sirtuins and PARP, the resulting flux from NAD to nicotinamide would be higher, and such cells might be hypersensitive to inhibition of nicotinamide salvage. Similarly, if maintenance of the malignant phenotype depends on activity of an NAD-consuming enzyme, then NAD depletion might rob a sirtuin or a PARP of its limiting substrate.

The redox functions of NAD are also sources of potential genotype-specific mechanisms. Cancer cells tend to rely less on oxidative metabolism than surrounding cells, largely because they are less driven by ATP production than production of nucleotides and lipids (Vander Heiden et al., 2009). A ramp up of glycolysis, a ramp down of citric acid cycle

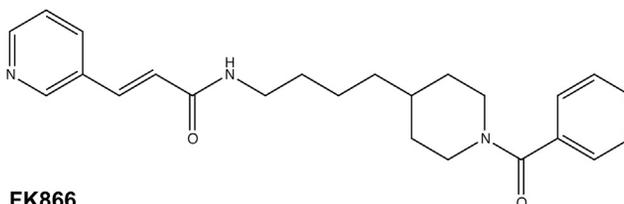
enzyme gene expression, and sensitivity to the glycolytic inhibitor 2-deoxyglucose have been reported for ALL cells (Boag et al., 2006). This would suggest that the NAD requirements for glucose fermentation, i.e., glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase, are targeted by Nampt inhibitors.

There is further potential for genotype-specificity in Warburgian models, because it has been shown that p53 mutations in ALL are largely restricted to cases with *MLL* rearrangement (Lanza et al., 1995). Ribose-5-phosphate synthesis through the pentose phosphate pathway (PPP), which is required for nucleotide biosynthesis, depends on cytosolic NADP and produces cytosolic NADPH for fatty acid synthesis and detoxification of reactive oxygen species. Strikingly, p53 inhibits glucose-6-phosphate dehydrogenase, such that p53-normal cells are inhibited in the use of glucose to generate NADPH, nucleotides and fatty acids, while

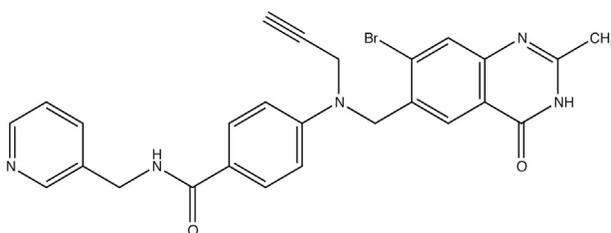
p53 mutant cells are derepressed for the PPP (Jiang et al., 2011). Consistent with inhibition of targets in glucose fermentation and the PPP by targeting NAD, FK866 is reported to lead to a sharp decline in nucleocytosolic NAD prior to affecting mitochondrial NAD pools (Pittelli et al., 2010). However, some NAD must be available for redox cycling in mitochondria to produce citrate for the subsequent appearance of cytosolic citrate and conversion to cytosolic acetyl-coA



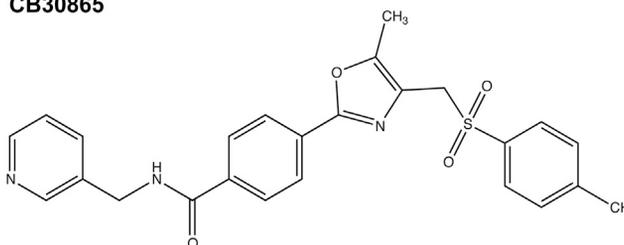
nicotinamide



FK866



CB30865



STF-118804

Figure 1. Chemical Structures of 3-pyridyl Compounds

for fatty acid synthesis (Ghanta et al., 2013).

Now that Nampt has been identified as an ALL target, further tumor profiling is warranted to identify the range of malignancies that are sensitive. An exciting possibility is that the therapeutic window for Nampt inhibitors can be modulated nutritionally; low NAD precursor vitamins might be warranted for the most aggressive chemotherapy, while provision of nicotinate or nicotin-

amide riboside might be warranted either to wash out from chemotherapy or to protect nonmalignant tissues from collateral damage (Bogan and Brenner, 2008).

REFERENCES

- Belenky, P., Bogan, K.L., and Brenner, C. (2007). *Trends Biochem. Sci.* 32, 12–19.
- Boag, J.M., Beesley, A.H., Firth, M.J., Freitas, J.R., Ford, J., Hoffmann, K., Cummings, A.J., de Klerk, N.H., and Kees, U.R. (2006). *Leukemia* 20, 1731–1737.
- Bogan, K.L., and Brenner, C. (2008). *Annu. Rev. Nutr.* 28, 115–130.
- de Boer, J., Walf-Vorderwülbecke, V., and Williams, O. (2013). *Leukemia* 27, 1224–1228.
- Druker, B.J. (2008). *Blood* 112, 4808–4817.
- Ghanta, S., Grossmann, R.E., and Brenner, C. (2013). *Crit. Rev. Biochem. Mol. Biol.* Published online September 19, 2013. <http://dx.doi.org/10.3109/10409238.2013.838204>.
- Jiang, P., Du, W., Wang, X., Mancuso, A., Gao, X., Wu, M., and Yang, X. (2011). *Nat. Cell Biol.* 13, 310–316.
- Lanza, C., Gaidano, G., Cimino, G., Coco Lo, F., Basso, G., Sainati, L., Pastore, C., Nomdedeu, J., Volpe, G., and Parvis, G. (1995). *Leukemia* 9, 955–959.

Matheny, C.J., Wei, M.C., Bassik, M.C., Donnelly, A.J., Kampmann, M., Iwasaki, M., Piloto, O., Solow-Cordero, D.E., Bouley, D.M., Rau, R., et al. (2013). *Chem. Biol.* 20, this issue, 1352–1363.

Muntean, A.G., and Hess, J.L. (2012). *Annu. Rev. Pathol.* 7, 283–301.

Pittelli, M., Formentini, L., Faraco, G., Lapucci, A., Rapizzi, E., Cialdai, F., Romano, G., Moneti, G., Moroni, F., and Chiarugi, A. (2010). *J. Biol. Chem.* 285, 34106–34114.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). *Science* 324, 1029–1033.