DRUG DISCOVERY

Malaria under arrest

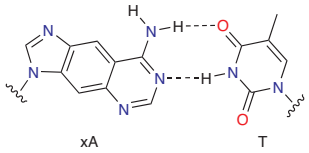
Science, published online 17 November 2011, doi:10.1126/science.1211936

The malaria parasites from *Plasmodium* species have both a liver stage (EEF or sporozoite stage) and a blood stage in human hosts. Because EEFs of certain *Plasmodium* strains can remain dormant for long periods and disease symptoms originate during the blood stage, therapeutics that can eliminate parasites of both stages are needed, yet most drugs target only the blood-stage parasites. To find drugs that might inhibit multiple parasite stages, Meister *et al.* screened a library of 5,697 compounds known to be active against *P. falciparum* blood stages. In a high-content imaging assay of sporozoite-infected cultured liver cells, the authors found 275 compounds that are structurally unrelated to known antimalarial scaffolds and decrease parasite size within cells after infection. They then used a hypergeometric mean function to determine which of the 2,715 independent chemical-scaffold clusters in the original library were fully represented among the hits, identifying quinazoline, pyrazolopyrimidine and imidazolopiperazine scaffold clusters as promising. A structure-activity relationship analysis of the imidazolopiperazine scaffold yielded compounds that are active against both parasite stages and have high potencies and desirable pharmacokinetic properties. By sequencing resistance mutations, the authors identified one target for three of the imidazolopiperazine compounds as an uncharacterized seven-transmembrane-domain–containing protein. As targets of other hits are also most likely distinct from known drug targets, as shown by the authors, these may represent new opportunities for eradicating malarial infections by arresting parasite development. *MB*

SYNTHETIC BIOLOGY

Cellular xDNA readers

J. Am. Chem. Soc. **133**, 18447–18451 (2011)

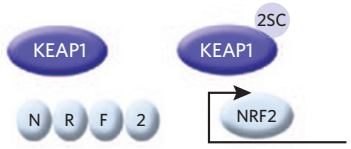


Synthetic nucleobase analogs have proven useful for developing non-natural DNA coding systems. For example, 'size-expanded DNA' (xDNA) nucleotides—in which purine or pyrimidine bases are structurally extended by a benzene ring fusion—retain their ability to pair with natural bases but form expanded double helices. DNA polymerases can replicate many alternative base pairs with reasonable fidelity *in vitro*, but completion of DNA synthesis is often impaired, limiting the cellular applications of synthetic genetic systems. Krueger *et al.* now show that xDNA modifications can undergo DNA replication in *Escherichia coli*. The authors created a series of tagged expression plasmids containing xDNA modifications in the coding region of a *GFP* gene. Transformation of *E. coli* with modified plasmids with up to four adjacent xDNA substitutions yielded green bacterial colonies; DNA sequencing revealed that, after transformation, xDNA templates are copied faithfully into plasmids with natural base pairs. Mutational analysis ruled out DNA repair pathway involvement in this conversion, but biochemical experiments showed that GFP production from xDNA templates was dependent on the copying of xDNA-containing plasmids by replicative and Y-family polymerases. Though additional experiments may reveal the mechanistic details of x-base replication, the current study highlights a new opportunity to explore alternative coding schemes in cells. *TLS*

CANCER

KEAPing NRF in check

Cancer Cell **20**, 511–523 (2011); *Cancer Cell* **20**, 524–537 (2011)



The Krebs cycle enzyme fumarate hydratase (FH) is mutated in a hereditary renal cancer syndrome, where *FH* mutation stabilizes expression of HIF-1α, a transcription factor that promotes cell survival in hypoxic conditions. Because other HIF-1α–stabilizing mutations lead to distinct tumor phenotypes, two groups set out to determine whether *FH* mutation could promote tumorigenesis independently of HIF1α. Using genetic approaches, Ooi *et al.* and Adam *et al.* now link disruption of *FH* with loss of activity of KEAP1, an electrophile-sensitive component of an E3 ubiquitin ligase, and increased activity of the antioxidant response transcription factor NRF2. Both groups demonstrated that the link between *FH* mutation and KEAP1 or NRF2 is independent of HIF-1α. Fumarate, the substrate for FH, accumulates in FH-deficient cells; to mimic this state, Ooi *et al.* treated cells with dimethyl fumarate and found increased expression of NRF2 and its target genes, suggesting that KEAP1, which is known to control the stability of NRF2, is sensitive to fumarate. MS in both studies confirmed the modification of several residues in KEAP1 to *S*-(2-succinyl)-cysteine (2SC) in fumarate-accumulating conditions. Ooi *et al.* further demonstrated that ubiquitinated KEAP1 was elevated in FH-deficient cells compared to controls; additional experiments using proteasomal inhibitors led to a model where 2SC-modified KEAP1 is ubiquitinated and degraded by the proteasome. This is the first HIF-1α–independent mechanism proposed to explain tumorigenesis resulting from *FH* mutation; however, it remains to be determined how activation of the NRF2 antioxidant-response pathway contributes to oncogenesis in hereditary renal cancer. *AD*

MICROBIAL ECOLOGY

When things go bad

J. Am. Chem. Soc. **133**, 18343–18349 (2011)

Small molecules often define the interactions between bacteria and other species. Seyedsayamdost *et al.* had previously identified the chemical components of a marine parasitic interaction, in which the microalga *Emiliania huxleyi* releases a lignin component—*p*-coumaric acid—into the environment, and the α-proteobacterium *Phaeobacter gallaeciensis* BS107 responds by producing two toxic roseobacticides. To explore the generality of this phenomenon, the authors tested the impact of *p*-coumaric acid along with four additional lignin components and related biosynthetic intermediates on roseobacticide production by *P. gallaeciensis* BS107. Four of the five lignin molecules elicited a chemical response, the components of which could be grouped into four families of roseobacticide analogs, including phenyl-, phenol- and indole-modified structures as well as two dimeric roseobacticides. The authors further observed that treatment with each of the four *E. huxleyi* elicitors yielded different combinations of the roseobacticides in different proportions. Extension of these tests to additional species identified *P. gallaeciensis* 2.10 as similarly responsive to the lignin elicitors with additional diversity in roseobacticide production: for *P. gallaeciensis* BS107, *p*-coumaric acid and sinapic acid yielded the strongest responses, whereas for *P. gallaeciensis* 2.10, ferulic acid and cinnamic acid were favored. These results help to define a parasitic system and stimulate further questions regarding the role of the individual roseobacticides. *CG*

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