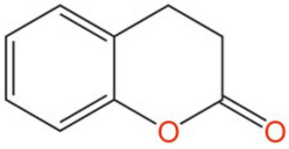
Un-sirtuin effects of natural products



The sirtuins are a family of NAD^+-dependent deacetylases with a range of substrates including p53, FOXO and histones. Mutational studies of the yeast sirtuin Sir2p and of two members of the human sirtuin family, SIRT1 and SIRT2, has implicated this family in determining life span, telomere maintenance and transcription of key apoptotic regulators. For this latter task, SIRT1 destabilizes p53 through deacetylation. When SIRT1 is downregulated or inhibited, p53 remains acetylated, enhancing its function in apoptosis. Activation of Sir2p by the red wine component resveratrol increases the life span of yeast. Olaharski, Rine and colleagues have exploited the fact that Sir2p is required for heterochromatic silencing at the yeast mating type loci to develop a chemical-genetic screen to find other modulators of the sirtuin family. Because they suspected that other natural products, like resveratrol, probably exist that inhibit sirtuins, the authors screened over 100 common environmental chemicals. This led them to dihydrocoumarin (DHC), a component of sweet clover that is used commercially in cosmetics, soaps and beverages. DHC, like an established Sir2p inhibitor, splitomicin, derepressed heterochromatic silencing in the yeast assay. DHC also inhibited human SIRT1 and SIRT2 *in vitro*. Remarkably, DHC caused an increase in p53 acetylation and in cell toxicity, as predicted for a SIRT1 inhibitor. It is not yet known if DHC can decrease the life span of model organisms, as might be expected, but this work certainly sheds light on how SIRT1 helps p53 in its role of balancing tumor suppression and stem cell depletion. It also raises the question of whether other environmental substances may influence lifespan. (*PLoS Genet.*, published online 16 December 2005, doi:10.1371/journal.pgen.0010077) *MB*

Cancer's invasive ingredient

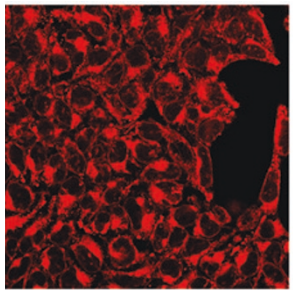
Cancer's ability to cause mortality is due, in part, to the ability of some tumor cells to become metastatic and migrate to other tissues in the body. One feature of metastatic transformation is the appearance of abnormal forms of cell-surface glycosphingolipids (GSLs), important mediators of cell-cell communication. In a recent publication, Kovbasnjuk, Donovan and coworkers now show that a GSL, globotriaosylceramide (Gb_3), is expressed three times more abundantly on metastatic colon tumor cells than on normal colon or nonmetastatic colon cancer cells. To examine the basis for the correlation between metastatic potential and expression of Gb_3, the authors localized Gb_3 within tumor cell culture models. In three different tumor cell types, they found that Gb_3-containing cells were located mainly at the edge of tumor cell islands on the surface of cells with filopodial protrusions. These cells, as well as Gb_3-containing Caco-2 cells, had a striking migratory phenotype, which could be blocked by RNAi knockdown of Gb_3 synthase expression. Consistent with a scenario whereby Gb_3 is necessary and sufficient to induce migration, Gb_3-negative cells transfected with Gb_3 synthase cDNA acquired the ability to migrate and to bind the B subunit of shiga toxin-1 (Stx1B), a known ligand and apoptotic mediator of Gb_3-positive cells. In mouse xenograph models of colon cancer, Stx1B could significantly reduce tumor growth, supporting a critical role for Gb_3-positive cells. Because the expression level of the Gb3-biosynthetic enzyme does not differ substantially between Gb_3-negative and Gb_3-containing cells, there must be an unidentified signal-transduction mechanism leading to Gb_3-mediated migration and metastasis. (*Proc. Natl. Acad. Sci. USA* **102, 19087–19092, 2005**) *GW*

Research Highlights written by Mirella Bucci, Terry L. Sheppard and Greg Watt.

Knocking off methyl groups

Epigenetic pathways, including the selective modification of histone proteins by acetylation or methylation, are important regulators of cellular gene expression. For example, the transcriptional activity of chromatin can be controlled by balancing the enzymatic addition or removal of methyl groups on specific lysine or arginine residues in histones. Although the transfer of methyl groups is a well-studied biosynthetic reaction, it has not been clear how the selective enzymatic removal of methyl groups is achieved. The recent characterization of demethylases suggests that these enzymes oxidatively cleave methyl groups from DNA or histone substrates to produce formaldehyde. Zhang and coworkers now report the isolation and characterization of a new family of broadly conserved histone demethylases. To identify candidate demethylases in HeLa cells, the authors developed a sensitive assay to detect formaldehyde. They identified JHDM1A, a new protein that has optimal demethylation activity in the presence of iron(II) and α-ketoglutarate cofactors. JHDM1A contains an essential JmjC domain, which had previously been generically assigned as a metal-binding motif associated with chromatin proteins. The authors demonstrated that JHDM1A demethylates Lys36 of histone H3 and shows enhanced specificity for the dimethylamine form of lysine, both *in vitro* and *in vivo*. Zhang and coworkers identified similar proteins in diverse eukaryotes and suggest that JmjC domains represent the signature motif for lysine demethylation activity throughout evolution. (*Nature*, **published online 18 December 2005, doi:10.1038/nature04433**) *TLS*

Making sense of copper ions



Christopher J Chang

Because of their prominent roles in enzyme active sites, copper ions are essential to living organisms. However, when cellular homeostasis of copper is disrupted, the concentration of intracellular copper ions may increase to toxic levels. Thus, quantitative measures of copper ion levels in cells aid the dissection of pathways of copper metabolism and pathology. Although selective probes for detecting copper ions have been developed previously, they require UV light treatment, which can damage living cells. In a recent article, Chang and colleagues described the development of a selective fluorescent probe to visualize copper in living cells. The authors conjugated a thioether-rich macrocyle to a fluorescent reporter to produce a water-soluble copper sensor, termed Coppersensor-1 (CS1). The 1:1 binding of Cu^+ ions to CS1 produced a ten-fold increase in fluorescence intensity focused at longer wavelengths. The authors demonstrated the selectivity of CS1 for Cu^+ in the presence of other metal ions, including Cu^{2+}, where the fluorescence emission profile was essentially unchanged. In living HEK293 cells treated with copper(II) chloride for several hours, CS1 staining produced an intense fluorescent image revealing high cytosolic Cu^+ ion levels, whereas in untreated cells or cells treated with copper metabolism in the presence of a Cu^+ chelator, fluorescence was diminished. This work should provide a useful tool for biologists investigating copper in living systems. (*J. Am. Chem. Soc.*, published online 9 December 2005, doi:10.1021/ja055064u) *GW*