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ANTICANCER DRUGS

Cutting the antioxidant supply chain

> Treatment with cyst(e)inase prevented tumour growth in multiple murine models

The reducing agent glutathione is particularly important to cancer cells, as the expression of oncogenes and the high rates of metabolism in these cells result in the production of excessive quantities of reactive oxygen species (ROS). Cramer *et al.* now report a novel therapeutic approach for the treatment of tumours that are associated with high ROS levels: they engineered an enzyme that depletes a key blood-borne antioxidant used by tumour cells. Administration of the enzyme inhibits tumour growth in multiple preclinical models.

The amino acid L-Cys is a precursor of glutathione, the key cellular metabolite that protects against ROS, as well as other antioxidants. Whereas normal cells produce sufficient L-Cys endogenously, oxidatively stressed cancer cells depend on L-Cys uptake from the blood. In the extracellular environment, L-Cys is predominantly present in its disulfide form, L-cystine (CSSC), and is taken up by cells primarily through the cystine/glutamate transporter (XCT). Using structure-guided mutagenesis, Cramer *et al.* engineered a human variant of cystathionine gamma-lyase (CGL) that degrades L-Cys and CSSC with high catalytic activity. Conjugation of the engineered enzyme to polyethylene glycol chains produced a therapeutic enzyme, which the authors named cyst(e)inase.

ROS production correlates with an aggressive phenotype in prostate cancer, and cyst(e)inase treatment decreased glutathione levels, increased ROS production and induced autophagy-like cell death in mouse and human prostate cancer cell lines. Treatment with cyst(e)inase prevented tumour growth in multiple murine models of allogenic or xenograft prostate cancer tumours as well as in breast cancer xenografts. Breast cancers are known to upregulate XCT and import CSSC, so they could be particularly susceptible to treatment with cyst(e)inase.

Chronic lymphocytic leukaemia (CLL) cells lack XCT and have been reported to be dependent on stromal production of L-Cys for their survival: the stromal cells take up CSSC and release L-Cys into the microenvironment, and the leukaemic cells then take up L-Cys using other transporter systems. Leukaemic B cells from a transgenic mouse model (*TCL1*-Tg:*p53*$^{-/-}$) that closely mimics an aggressive form of human CLL were susceptible to cyst(e)inase treatment, even when co-cultured with stromal cells. Primary leukaemic cells isolated from individuals with CLL were also susceptible to cyst(e)inase treatment, whereas B cells from healthy donors were not, which suggests that cyst(e)inase could preferentially kill metabolically hyperactive cells.

In long-term experiments, *TCL1*-Tg:*p53*$^{-/-}$ mice that were treated twice per week with cyst(e)inase from the age at which the disease first manifests (2 months) survived for a median of 7 months with no signs of toxicity. By comparison, *TCL1*-Tg:*p53*$^{-/-}$ mice treated with fludarabine, which is the current standard treatment for CLL, survived for 5.3 months, and untreated mice survived for 3.5 months. Toxicity was also not observed in non-human primates that were treated with cyst(e)inase.

Cancer cells have long been known to be more metabolically active than their normal counterparts, and most cancer cells show elevated levels of ROS. Treatment with cyst(e)inase could be a novel way to target such cancers by disabling their antioxidant defence mechanisms.

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ORIGINAL ARTICLE Cramer, S. L. *et al.* Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. *Nat. Med.* http://dx.doi.org/10.1038/nm.4232 (2016)