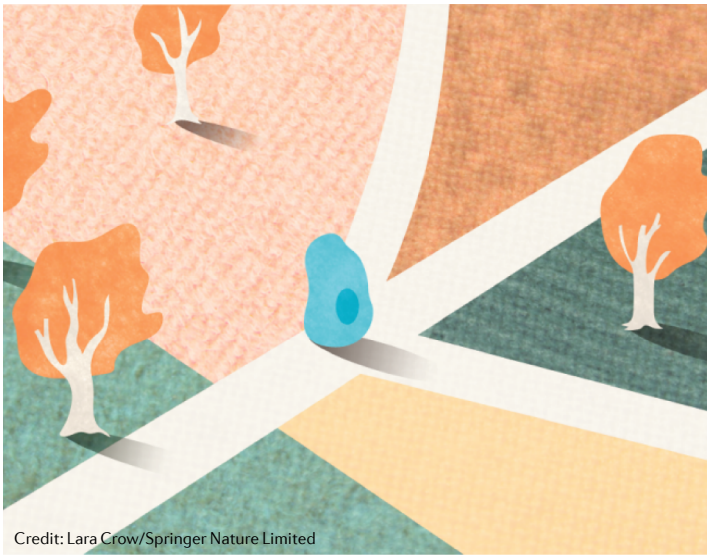
CANCER METABOLISM

Context determines which pathway to use for NAD synthesis



Credit: Lara Crow/Springer Nature Limited

Nicotinamide adenine dinucleotide (NAD) is a coenzyme for redox reactions in metabolic pathways required for survival and growth in normal tissues and cancer. It can be sourced from three different pathways, including the Preiss–Handler pathway (PHP), generating NAD from nicotinic acid; the de novo synthesis pathway (DNP), generating NAD from tryptophan; and the salvage pathway (SP), generating NAD from nicotinamide (NAM). Chowdry et al. show that tissue context determines the metabolic pathway by which NAD is produced in cancer, resulting in different metabolic dependencies that can be harnessed in precision oncology.

To identify differential roles of the three NAD synthesis pathways, the authors analysed the mutation and copy number status of genes encoding the rate-limiting enzymes of each pathway (nicotinate phosphoribosyltransferase (NAPRT) for the PHP, quinolinate phosphoribosyltransferase for the DNP and nicotinamide phosphoribosyltransferase (NAMPT) for the SP). While mutations in the respective genes were found in less than 1% of the samples, *NAPRT* copy numbers were increased in a range of cancer types including ovarian cancer, pancreatic cancer and prostate cancer, whereas *NAMPT* copy numbers were increased in different cancer types including lung cancer, gastric cancer and oesophageal cancer, and to a lesser degree than that seen with *NAPRT*. Interestingly, the amplification of *NAPRT* and/or *NADSYN1*, a gene encoding another enzyme in the PHP pathway, was correlated with high expression of these genes in the tissue from which the respective cancer arose, indicating a role for lineage dependency and tissue context in determining which metabolic pathway is used to produce NAD.

The authors chose to focus on the PHP and SP for further analysis. In a panel of 54 cancer cell lines, 29 had amplified *NAPRT* and/or *NADSYN1*. Short hairpin RNA (shRNA) targeting rate-limiting enzymes of the PHP or the SP led to reduced survival and NAD levels in PHP-amplified cancer cells only when *NAPRT* was targeted, compared with cells targeted with *NAMPT* shRNA or control shRNA. The survival and NAD levels of non-PHP-amplified cancer cells were only sensitive to the loss of *NAMPT*. Non-cancer cells were not sensitive to *NAPRT* or *NAMPT* loss, indicating that these cells can maintain NAD synthesis by using multiple pathways.

Further analysis using chromatin immunoprecipitation followed by sequencing revealed a *NAMPT* enhancer region with epigenetic marks including H3K27 acetylation resulting in higher chromatin accessibility in non-PHP-amplified cancer cells compared with PHP-amplified cancer cells or non-cancer cells. This enhancer region was responsible for controlling *NAMPT* expression and NAD levels in non-PHP-amplified cancer cells and was required for their survival.

To compare the contribution of PHP or SP enzyme expression in PHP-amplified or non-PHP-amplified cancer cell lines in vivo, *NAPRT*-amplified OV4 ovarian adenocarcinomas were treated with doxycycline (Dox)-inducible shRNA targeting *NAPRT*, *NADSYN1*, *NAMPT* or *NMRK1* (encoding another SP enzyme) and implanted into the left flanks of mice. Non-PHP-amplified H460 lung cancer cells that were treated with the same respective shRNAs were implanted into the opposite flanks. Upon Dox treatment a week after implantation, OV4 but not H460 tumours without *NAPRT* or *NADSYN1* expression did not grow further and durably regressed, while intratumoural NAD levels were reduced. By contrast, loss of *NAMPT* or *NMRK1* did not affect OV4 tumour growth or NAD levels compared with tumours treated with control shRNA. H460 tumours with loss of *NAMPT* or *NMRK1* grew slower and had lower levels of intratumoural NAD compared with control shRNA.

> “amplification of *NAPRT* and/or *NADSYN1* … correlated with high expression of these genes in the tissue from which the respective cancer arose”

This differential dependency on the PHP or SP was reflected in experiments using small molecules to inhibit NADSYN1 (NADSYNi) or NAMPT (FK-866). The growth and intratumoural NAD levels of OV4 tumours but not of H460 tumours were sensitive to NADSYNi; however, FK-866 did not affect OV4 tumour growth, but inhibited H460 tumour growth.

These findings highlight the metabolic heterogeneity between tumour tissues based on gene amplification and epigenetic remodelling, and open up opportunities for differential targeting of a cofactor that is central to maintaining metabolic and cellular homeostasis in cancer.

Ulrike Harjes

ORIGINAL ARTICLE Chowdhry, S. et al. NAD metabolic dependency in cancer is shaped by gene amplification and enhancer remodelling. *Nature* https://doi.org/10.1038/s41586-019-1150-2 (2019)