COMMENTARY

Linking hyperosmotic stress and apoptotic sensitivity

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Cellular responses to hypertonic stress and how these are linked to the induction of or sensitisation to cell death signals are incompletely understood and rarely studied in cancer. Using cell lines derived from head and neck squamous cell carcinoma (HNSCC), Heimer et al. demonstrate that hypertonic environments neutralise the antiapoptotic Bcl-2 family member Mcl-1 by upregulating its antagonist Noxa. Consequently, hypertonically stressed HNSCC cells rely solely on Bcl-xL for survival and succumb to apoptosis when challenged by pharmacological Bcl-xL inhibition. Similar findings were reported in colorectal cancer cells in related manuscripts, suggesting that a common and conserved mechanistic link might exist between hyperosmotic stress and cellular sensitisation to apoptosis.

The human body tightly controls tonicity, with already rather small increases in extracellular fluid tonicity (a hypertonic environment) triggering thirst reflexes. Hypertonicity, for example due to elevated extracellular Na^+ concentrations (hypernatraemia), generates an osmotic pressure gradient across the cell membrane that causes cells to lose water and volume. Such cells experience intense stress, which in extreme cases results in loss of function and cell death. Consequently, cells exposed to locally high concentrations of solutes in the extracellular space and rapid changes in tonicity, such as cells of the renal medulla, can engage complex stress compensation and survival responses [1].

The consequences of hypertonic stress on cancer cells and how these might alter cell death susceptibility and treatment responsiveness are rarely studied. A notable exception is the group of Martin Ehrenschwender and colleagues, who analyse apoptosis susceptibility at isotonic and hypertonic conditions in cellular models of various cancers. In their latest work (Heimer et al., [2]), they demonstrate that head and neck squamous cell carcinoma (HNSCC) cells respond to hypertonic stress by inactivating the protein Mcl-1. Mcl-1 is an antiapoptotic member of the Bcl-2 protein family and, together with Bcl-xL and Bcl-2 itself, prevents the permeabilisation of the outer mitochondrial membrane by Bax and Bak, two pore-forming proteins that initiate the execution phase of apoptosis [3]. Indeed, eliminating antiapoptotic Bcl-2 family members suffices to spontaneously induce the mitochondrial apoptosis pathway, presumably by intrinsic Bax autoactivation [4]. Conversely, high expression of any of these proteins can limit the success of cytotoxic anticancer therapies [5].

Heimer et al. demonstrate that HNSCC cells rely on both Mcl-1 and Bcl-xL expression for survival, since combination treatment with S63845 (Mcl-1 inhibitor) and WEHI-539 (Bcl-xL inhibitor) but not single agent

Abbreviations
EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; HNSCC, head and neck squamous cell carcinoma.

treatment invariably resulted in cell death. Interestingly, when subjecting these cells to hypertonic stress by increasing the extracellular NaCl concentration, Mcl-1 dependency was lost and Bcl-xL inhibition alone sufficed to induce apoptosis (Fig. 1). Elevating tonicity by trehalose provided similar results, indicating that hypertonic stress factors might universally shift cells towards Bcl-xL dependency for survival. In a closely related recent manuscript focusing on colorectal cancer cells, the authors observed very similar responses [6], indicating that the neutralisation of Mcl-1 upon hypertonic stress is conserved beyond the HNSCC setting. Bcl-xL dependency also manifested when growing cells in low-oxygen environments and when analysing 3D spheroid models, indicating the relevance of the hypertonicity response in cell culturing scenarios that are closer to *in vivo* conditions.

Heimer *et al*. then set out to answer the important question on how the activity of Mcl-1 is neutralised. Among the multidomain Bcl-2 family members, Mcl-1 is known for its high turnover, so that altered production or degradation rates could be obvious and immediate modes of regulation. However, the amounts of Mcl-1 transcripts and protein, as well as the Mcl-1 half-time, remained unaffected. Instead, the authors noted that the BH3-only protein and Bcl-2 family

member Noxa, the most prominent antagonist of Mcl-1 [7], accumulated in response to hypertonic stress. In contrast, the amounts of all other studied family members remained stable or decreased. Since Mcl-1 dependence was restored upon depleting Noxa expression but not when targeting Bim, an alternative BH3-only protein expressed in substantial amounts in HNSCC cells, the crucial role of Noxa in this setting could be confirmed.

But what links hypertonicity to Noxa accumulation? Hypertonicity can induce widespread intracellular protein aggregation and endoplasmic reticulum (ER) stress, and both autophagic and proteasomal clearance of aggregates and misfolded proteins contribute to establishing tolerance to hypertonicity [8,9]. Heimer *et al*. noted that hypertonic stress induced acute release of ER Ca^{2+} stores, which could be expected to additionally contribute to the manifestation of ER stress and proapoptotic signalling responses. However, Ca^{2+} signalling in the hypertonicity scenario surprisingly appeared to be a cell-protective response. Furthermore, hypertonically stressed cells failed to induce ATF4 signalling, which, during ER stress, promotes Noxa transcription [10]; neither was the related ATF6 signalling axis required for Noxa accumulation. These results therefore question whether HNSCC cells experience notable ER stress at all under the conditions studied by the authors. While it currently seems unlikely that ER stress and the unfolded protein response are major factors in upregulating Noxa upon hypertonicity, such a conclusion might be premature. Further analysis of ER stress hallmarks, such as BiP/GRP-78 accumulation or CHOP induction [11], and associated mechanistic links to Noxa induction remain unstudied so far. It might also be worthwhile to analyse whether more immediate links exist between hypertonic stress and Mcl-1 neutralisation. Hypertonicity can cause spontaneous and swift protein aggregation, (temporary) overload of cellular proteolytic capacity and compensatory induction of autophagy [8,12]. Overload of the proteasome possibly resembles stress conditions that arise when challenging cells with proteasome inhibitors. Proteasome inhibition induces Noxa accumulation, both by impairing its degradation and by active transcriptional induction [13], with the latter depending on p53. The authors indeed conducted experiments in which they knocked down p53 expression and studied viability loss in PCI-68 cells. p53 depletion conferred notable protection of hypertonically stressed cells across all Bcl-xL inhibitors tested in one of these experiments, but the authors for now excluded a role of p53 due to irreproducibility of this trend in a second experiment. Overall, whether
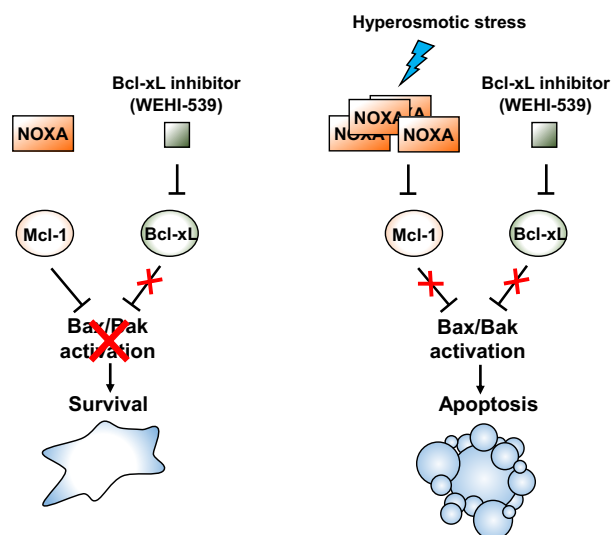

Fig. 1. Combining hypertonic stress with Bcl-xL inhibition drives HNSCC cells into apoptosis. HNSCC cells resist apoptosis upon Bcl-xL inhibition due to the expression of Mcl-1, an antiapoptotic Bcl-2 family member. However, hyperosmotic stress due to hypertonic environments increases the expression of Noxa, an antagonist of Mcl-1. As a consequence, cells exclusively depend on Bcl-xL for survival and succumb to apoptosis when treated with the Bcl-xL inhibitor WEHI-539.

protein aggregation, proteasome overload and to some extent possibly also p53 play a role in altering Noxa amounts upon hypertonic stress therefore warrants further formal investigation. Notably, sublethal proteasome inhibition was already reported to establish susceptibility to Bcl-2/Bcl-xL inhibition by Noxa-dependent neutralisation of Mcl-1 in colon cancer cells [14]. It will therefore be interesting to see whether this signalling context plays a role in linking hypertonic stress to Bcl-2 family signalling and apoptosis sensitisation.

The primary treatment options for HNSCC patients include chemoradiotherapy with high doses of cisplatin and targeting of the epidermal growth factor receptor (EGFR) with antagonistic antibodies [15]. Heimer et al. therefore compared the efficacy of radiotherapy and EGFR-targeting via erlotinib to the combination of hypertonicity/Bcl-xL inhibition in HNSCC cells. Combining hypertonic stress and Bcl-xL inhibition more potently eliminated HNSCC cells than treatment with Erlotinib or radiation. However, translational avenues by which the apoptosis-sensitising effects of hypertonicity could improve anticancer treatments still need to be defined. While the authors speculate about delivering osmotically active solutes to the tumour site or to implant devices that release such solutes, it should also be noted that hypernatraemia can manifest as a side-effect of treatments with various FDA-approved drugs, such as lithium or the antibiotic demeclocycline [16]. It thus might be worthwhile assessing whether such medications can be repurposed to induce acute hypertonic stress and whether concomitant Bcl-xL antagonism would cause systemic toxicities. Since platelets depend on Bcl-xL for survival and Bcl-xL inhibitors induce thrombocytopenia, the therapeutic window for Bcl-xL inhibitors in such settings will need to be evaluated carefully. Furthermore, the long-term adaptation to hypertonic stress will require further attention, since Heimert et al. found that Noxa accumulated only transiently (< 24 h), and consequently, cell death sensitisation might likewise be transient. Irrespective of translational relevance, the findings reported by Heimer et al. contribute to a better cell biological understanding of stress responses arising from hypertonicity. This fascinating field of study surely offers ample opportunities to further decipher how cells adjust their thresholds for death and survival decisions during hypertonic stress over the coming years.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

Author contributions

DS and MR wrote the manuscript.

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