RESEARCH HIGHLIGHT

Triaptosis: an endosome-dependent cell death modality

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Recent research published in *Science* reveals that menadione, a precursor to vitamin K, induces a unique cell death pathway called triaptosis by oxidizing phosphatidylinositol 3-kinase PIK3C3/VPS34. This oxidation leads to endosomal dysfunction, highlighting how excessive oxidative stress can compromise cellular structures and activate specific cell death pathways.

Vitamin K is essential for blood coagulation and bone mineralization and may also mitigate pathological calcium deposition in soft tissues, organs, and vascular structures, particularly in individuals with elevated risk factors, such as chronic kidney disease, cardiovascular disease, and diabetes.[1] Recent research by Swamynathan et al. identifies menadione (also known as vitamin K3) — a synthetic precursor to vitamin K2 — as a pro-oxidant with promising therapeutic potential in prostate cancer as well as X-linked myotubular myopathy, a fatal hereditary disease in boys caused by mutations in the myotubularin 1 (*MTM1*) gene on the X chromosome.[2] This effect is mediated through a unique cell death mechanism termed triaptosis, characterized by endosomal dysfunction (Fig. 1).

Historically, antioxidants were hypothesized to protect against cancer by neutralizing reactive oxygen species (ROS), thereby preventing DNA damage and mutation.[3] However, multiple large-scale, randomized clinical trials have failed to support this hypothesis. The foundational concept of the current study builds on the findings from the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which associated antioxidant supplements such as vitamin E (400 IU/day of all rac-α-tocopheryl acetate) and selenium (200 μg/day from L-selenomethionine) with an increased risk of prostate cancer in humans.[4] Swamynathan et al. proposed that pro-oxidants, in contrast, may serve a therapeutic purpose. They focused on menadione because previous studies have shown that, under certain conditions, menadione can induce oxidative cell death.[5,6] Specifically, their study demonstrates that supplementation of menadione at 0.15 mg/mL in drinking water significantly slowed prostate cancer progression in a genetically engineered mouse model ($Pten^{loxP/loxP}$;$Trp53^{loxP/loxP}$) over 18 weeks. This approach was well-tolerated, with no effects on coagulation, and provided longer antitumor effects compared to androgen deprivation therapy, the first-line treatment of symptomatic metastatic prostate cancer. Further in vitro experiments across 100 cancer cell lines demonstrated that those with mutations or depletion in Kelch-like ECH-associated protein 1 (KEAP1) — which enhance the stability of NFE2-like bZIP transcription factor 2 (NFE2L2) and strengthen the antioxidant response — showed increased resistance to menadione. These findings suggest that intrinsic redox-buffering capacity may impact therapeutic efficacy.

Glutathione (GSH) is a small antioxidant molecule found in most cells, critical for protecting cells against oxidative stress and maintaining redox balance.[7] The authors further demonstrated that GSH depletion is critical to menadione's cytotoxicity; however, the underlying mechanism driving this depletion — such as impacts on cystine uptake or inhibition of key enzymes in GSH synthesis — remains unidentified. Given the involvement of GSH in various cell death processes, including apoptosis, necroptosis/programmed necrosis, and ferroptosis,[8] they compared the effects of menadione with those of staurosporine, hydrogen peroxide, and erastin in the presence or absence of specific inhibitors, such as Z-VAD-FMK (pan-caspase inhibitor), Q-VD-Oph (pan-caspase inhibitor), DPQ (poly(ADP-ribose) polymerase 1 (PARP1) inhibitor), BAPTA-AM (calcium chelator), desferoxamine (iron chelator), or ferrostatin-1 (radical-trapping antioxidant). Although menadione-induced transcriptomic changes mirrored those of erastin, none of the standard cell death inhibitors mitigated menadione's cytotoxic effects. Menadione also differed from other oxidative stress-related compounds targeting the mitochondria (phenformin), lysosomes (chloroquine), and the nucleus (doxorubicin). Functional assays further revealed that menadione did not trigger key markers associated with apoptosis (mitochondrial ROS production and caspase activation), autophagy (accumulation of lipidated microtubule associated protein 1 light chain 3 (MAP1LC3)-II), or ferroptosis (lipid ROS accumulation). However, morphological analyses indicated significant cytoplasmic vacuole accumulation in menadione-treated cells, suggesting that it may trigger a unique oxidative cell death pathway.

Mechanistic insights from a whole-genome CRISPR screen in human metastasis-derived PC3 cell line, combined with bioinformatic analyses, suggested that menadione disrupts endocytosis, a process by which cells internalize external substances (e.g., nutrients). Specifically, gene set enrichment analysis revealed that blocking the early stages of endocytosis and endosome formation sensitized cells to menadione, whereas inhibiting late endosomal progression diminished its efficacy. Importantly, menadione was found to directly target phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3, also known as VPS34), a class III phosphoinositide 3-kinase essential for endosome formation and intracellular sorting, through the oxidation of cysteine residues

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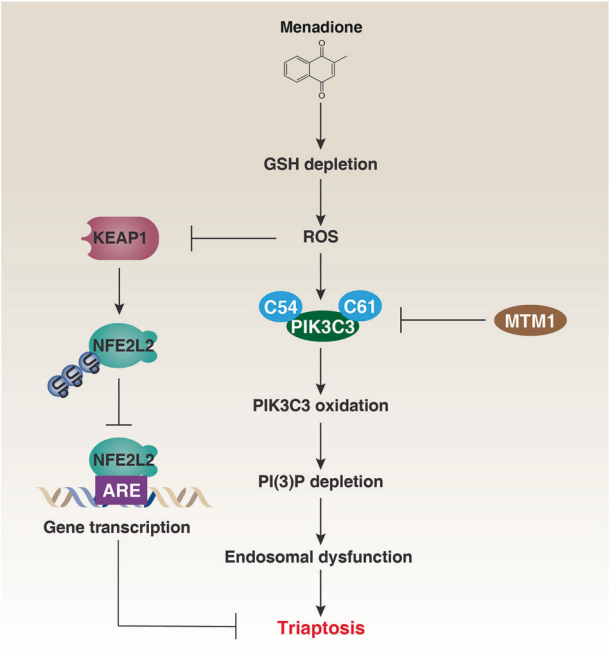

Fig. 1 Mechanism of triaptosis. Menadione, a fat-soluble vitamin precursor, induces GSH depletion and generates ROS, leading to the oxidation of cysteine residues (Cys54 and Cys61) on PIK3C3. This oxidation results in PI(3)P depletion, causing endosomal dysfunction and triggering triaptosis. As a cellular defense mechanism, NFE2L2 activation is achieved through inhibition of KEAP1-mediated ubiquitin-proteasome degradation. This stabilization of NFE2L2 promotes its nuclear translocation, where it binds to antioxidant response elements (AREs) and drives the expression of genes involved in oxidative stress mitigation. Beyond inhibiting tumor growth, such as in prostate cancer, menadione also has therapeutic potential in treating X-linked myotubular myopathy, a fatal hereditary disease in boys caused by mutations in the *MTM1* gene on the X chromosome. MTM1 is a phosphatase that antagonizes PIK3C3 kinase activity.

(Cys54 and Cys61). This oxidative interference leads to depletion of phosphatidylinositol 3-phosphate (PI(3)P), a hallmark lipid of early endosomes, causing endosomal dysfunction in prostate cancer cells. This disruption in PI(3)P results in membrane blebbing and rupture, defining the sequence of events that comprise triaptosis.

PIK3C3 kinase is essential for autophagosome formation via complex I (PIK3C3–PIK3R4–ATG14 (autophagy related 14)–BECN1 (beclin 1)) and for the establishment of the endosomal compartment via complex II (PIK3C3–PIK3R4–UVRAG (UV radiation resistance-associated gene)–BECN1–nuclear receptor binding factor 2 (NRBF2)).[9] Notably, PIK3R4 (also known as VPS15) emerged as one of the top sensitizing hits in this whole-genome CRISPR screen. Furthermore, bioinformatic analysis of 17,199 genes, ranked by correlation with *PIK3R4* essentiality in 1095 cancer cell lines, revealed that complex II components (e.g., *UVRAG*) had a stronger genome-wide correlation with PIK3R4 than complex I components (e.g., *ATG14*). However, the roles of UVRAG and ATG14 in triaptosis remain unexamined, though menadione inhibits chloroquine-induced MAP1LC3-II accumulation.

The therapeutic potential of menadione extends beyond oncology, as demonstrated in a model of an X-linked myotubular myopathy caused by the absence of MTM1, the phosphatase that antagonizes PIK3C3. This genetic disorder, characterized by defective endosomal sorting due to unregulated PI(3)P production, benefits from the menadione-mediated inhibition of PIK3C3, which compensates for the absence of *MTM1* and restores PI(3)P balance. In *Mtm1*-deficient male C57BL/6J mice, dietary supplementation with menadione improved muscle development and doubled survival to 62 days highlighting the therapeutic potential of menadione in non-oncological indication.

In summary, Swamynathan et al. provide novel insights into the pro-oxidant and pharmacological properties of a vitamin K derivative. By elucidating the capacity of menadione to block PIK3C3, this study opens potential treatment avenues for prostate cancer, myotubular myopathy, and other PI(3)P dysregulation-related conditions. Although the study suggests the capacity of NFE2L2 to enhance resistance to menadione, the specific NFE2L2-target genes responsible for these effects remain to be identified. Additionally, while the study introduces a novel oxidative cell death pathway, its prevalence in human tumors remains uncertain. Future research should investigate whether triaptosis has therapeutic potential in cancer patients. Given the critical role of endosomal sorting in antigen presentation and immune cell activation, examining the immunogenicity of triaptosis is another intriguing prospect.[10]

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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