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L-asparaginase-induced apoptosis in ALL cells involves IP_3 receptor signaling

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ABSTRACT

L-asparaginase treatment is used in the clinic to treat acute lymphoblastic leukemia (ALL) patients. Lee et al. (2019, Blood 133:2222-2232) demonstrated that L-asparaginase induces apoptosis by activating inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} signaling in a Huntingin-associated protein 1 (HAP1)-dependent manner. Moreover, HAP1 levels inversely correlate with the sensitivity of the ALL cells to L-asparaginase. HAP1 can therefore be used as biomarker for evaluating L-asparaginase resistance.

Recent work has highlighted that selectively suppressing pro-apoptotic intracellular Ca^{2+} signals favors the survival of various cancer types, including hematological cancers. The relation between Ca^{2+} signaling and cell survival versus cell death is also evident from the fact that both oncogenes and tumor suppressors modulate intracellular Ca^{2+} signaling by directly acting on key proteins responsible for intracellular Ca^{2+} homeostasis [1]. One of these key proteins is the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), a ubiquitously expressed Ca^{2+}-release channel residing at the endoplasmic reticulum (ER) and known to be regulated by a plethora of associated proteins [2]. This channel plays a major role in intracellular Ca^{2+} signaling in general as well as in Ca^{2+} transfer from the ER to the mitochondria in particular. In this respect, high IP_3R activity is linked to cell death. One of the mechanisms of action of the well-studied anti-apoptotic Bcl-2 protein is therefore suppressing excessive IP_3R-mediated Ca^{2+} release thereby preventing cancer cell death [3].

A novel role for Ca^{2+} signaling is presented in a recent publication on acute lymphoblastic leukemia (ALL) [4]. The current clinical treatment of pediatric ALL patients as well as of adolescents and young adults generally involves intensive systemic delivery of L-asparaginase [5]. The rationale for the use of this enzyme is that ALL cells express low levels of asparagine synthetase and therefore depend on the uptake of extracellular asparagine for protein synthesis. The presence of L-asparaginase however depletes plasma asparagine leading to a shortage of the amino acid, thereby driving ALL cells to their death. However, the treatment itself can be a source of problems for the patient: L-asparaginase, besides triggering allergic reactions, may lead to high-grade toxicities, including hepatotoxicity and acute pancreatitis. Moreover, a significant number of patients relapse after developing resistance to the treatment [5].

The development of acute pancreatitis has already been linked to the activation, by L-asparaginase, of the proteinase-activated receptor 2 of pancreatic acinar cells [6]. This leads to phospholipase C activation, production of the intracellular messenger IP_3 and consequently to Ca^{2+} release via the IP_3R. The increase in cytosolic Ca^{2+} concentration is further amplified by subsequent store-operated Ca^{2+} entry (SOCE) and a reduced Ca^{2+}-extrusion activity. The high and persistent increase in cytosolic [Ca^{2+}] provokes premature activation of pancreatic digestive enzymes and the onset of acute pancreatitis, a potentially lethal condition [6].

Rather unexpectedly, it was recently shown [4] that also the development of resistance to L-asparaginase treatment is related to changes in the intracellular handling of Ca^{2+}. The link between resistance to L-asparaginase and Ca^{2+} is through the Huntingtin-associated protein 1 (HAP1). As the name indicates, HAP1[1] was the first described binding partner of the protein Huntingtin (Htt), which polyglutamine expansion causes the autosomal-dominant neurological disorder known as Huntington's disease [7]. HAP1 is abundantly expressed in the brain and its interaction with Htt is increased after expansion of the polyglutamine sequence located in the N-terminus of the latter (Htt^{exp}). In brain, a ternary complex is formed between the type 1 IP_3R, Htt (or Htt^{exp}) and HAP1 [8]. The type 1 IP_3R is sensitized by

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[1] In rodents, two HAP1 splice isoforms exist, HAP1A and HAP1B. As the human HAP1 is most closely related to the rodent HAP1A, it is in the literature also sometimes indicated as HAP1A.

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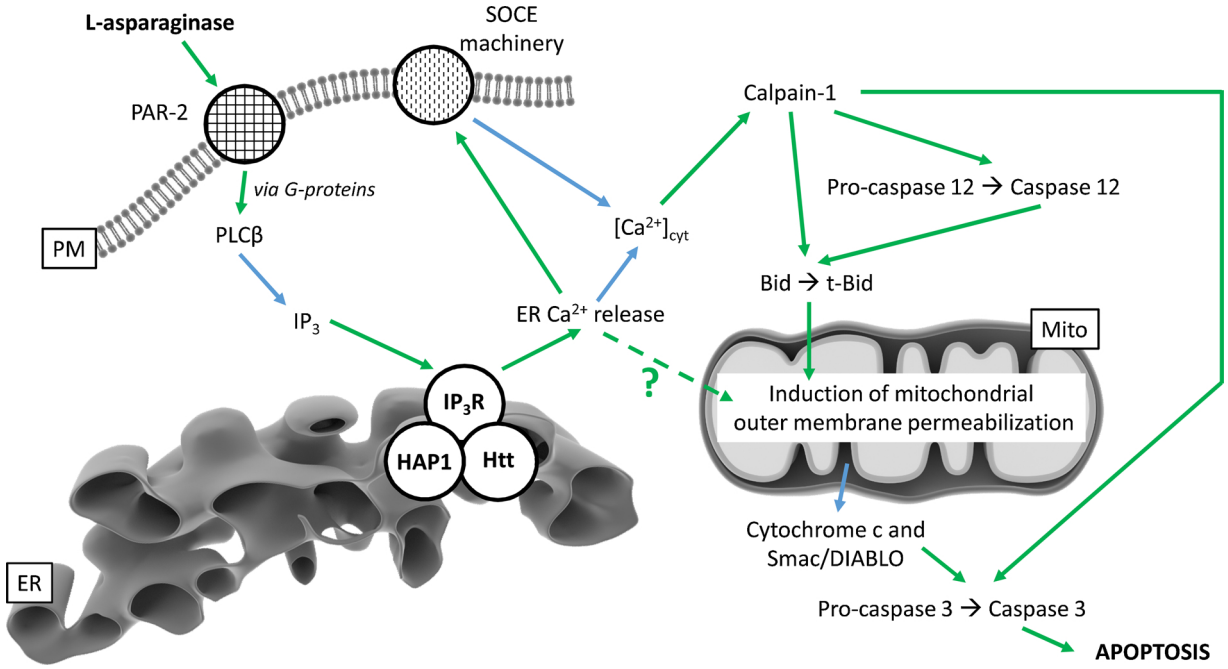

Fig. 1. Representation of the proposed signaling pathway by which L-asparaginase treatment triggers intracellular Ca^{2+} signaling and apoptosis in ALL cells, based on the work of Lee et al. (2019, Blood 133:2222-2232) [4]. Plasma membrane (PM), endoplasmic reticulum (ER) and mitochondria (Mito) are indicated. HAP1 appears to be needed to sensitize the IP_3R to Htt (formation of a ternary complex) in order to trigger Ca^{2+}-dependent apoptosis. Moreover, the IP_3-induced Ca^{2+} release from the ER can be amplified by store-operated Ca^{2+} entry (SOCE) from the extracellular milieu, further contributing to apoptosis induction. Cancer cells lacking HAP1 therefore fail to engage increased Ca^{2+} signaling, avoiding apoptosis induction in response to L-asparaginase treatment. The key elements of this process are indicated in bold. Stimulatory processes are represented by green arrows, and production or release of intracellular messengers by blue arrows. The role of Ca^{2+} transfer from the ER to the mitochondria in this process however remains to be elucidated (dashed arrow). Abbreviations used: $[Ca^{2+}]_{cyt}$, cytosolic Ca^{2+} concentration; ER, endoplasmic reticulum; HAP1, Htt-associated protein 1; Htt, Huntingtin; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; PAR-2, proteinase-activated receptor 2; PLCβ, phospholipase C β; SOCE, store-operated Ca^{2+} entry.

either Htt^{exp} alone or by the wild-type Htt in the presence of HAP1. Subsequent work with murine HAP1-knockout cells demonstrated that HAP1 plays a crucial role in linking Htt (or Htt^{exp}) to the type 1 IP_3R, sensitizing this channel and thus augmenting IP_3-induced Ca^{2+} release [9]. Moreover, as depletion of the ER Ca^{2+} stores is coupled to subsequent SOCE, HAP1 will thus also support an increased SOCE. The additional upregulation of Stim 2, an ER-resident protein participating in the activation of SOCE, may thereby contribute to the increased Ca^{2+} entry [10].

The new study [4] indicates that HAP1 also plays an important physiological role in regulating intracellular Ca^{2+} handling in leukocytes by participating in a complex consisting of IP_3R, Htt and HAP1. L-asparaginase induces Ca^{2+} release from the ER and Ca^{2+}-dependent apoptosis in SEM cells, a B cell line derived from a relapsed pediatric ALL patient. Lowering the levels of HAP1 reduced, for a currently unknown reason, slightly the ER Ca^{2+} store content. Furthermore, HAP1 deficiency led to inhibition of L-asparaginase-mediated IP_3-induced Ca^{2+} release and diminished SOCE, thereby suppressing apoptosis induction. These data demonstrate a tight coupling between the expression of HAP1 and the ability of L-asparaginase to induce apoptosis via IP_3R-mediated Ca^{2+} release. Although the mechanism involved was not fully elucidated at the functional and molecular level, it was suggested that HAP1 sensitizes the IP_3R. This makes the cells more prone to Ca^{2+}-dependent apoptosis, likely via the consecutive activation of the Ca^{2+}-dependent protease calpain-1 and of caspase 12 (Fig. 1). When active, these proteases cleave Bid and pro-caspase 3, producing their pro-apoptotic forms t-Bid and caspase 3 respectively.

The various patient ALL cells investigated in the study all expressed HAP1, though their expression levels varied tenfold. Interestingly, HAP1 levels appeared to inversely correlate with the measured IC_{50} for L-asparaginase. Moreover, knockdown of HAP1 led to L-asparaginase resistance, but did not affect the sensitivity of the cells to doxorubicin, another pro-apoptotic drug to which ALL cells are sensitive. This suggests a specific role for HAP1 in L-asparaginase-induced apoptosis in comparison to doxorubicin [4]. This result supports the unbiased genome-wide RNAi screening for 24,000 shRNAs performed by the authors, indicating that HAP1 levels may be considered in ALL as a biomarker for L-asparaginase resistance, which would allow stratificating the patients and adapting their therapeutic regimen.

Further work will now be needed to fully understand the role of HAP1 on apoptosis induction via increased Ca^{2+} signaling. It will be crucial to evaluate, in a quantitative manner, the effect of HAP1 on ER Ca^{2+} store levels and IP_3-induced Ca^{2+} release as well as on the subsequent SOCE activation. Another important aspect for future studies will be to measure whether HAP1 affects Ca^{2+} transfer between ER and mitochondria as mitochondrial $[Ca^{2+}]$ is known to control mitochondrial function, including bioenergetics and the occurrence of autophagy or apoptosis [3].

In conclusion, the already available data [4] indicate on the one hand an unexpected role for HAP1 in intracellular Ca^{2+} handling in non-neuronal cells and on the other hand highlight the important role that intracellular Ca^{2+} signaling pathways play in cell survival versus cell death decisions. Moreover, HAP1 mutations and deletion also occur in other cancer types, albeit at relatively low frequencies. Taken together these data underscore the importance of intracellular Ca^{2+} signaling pathways in cancer and designate them as potentially useful therapeutic targets.

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Declaration of Competing Interest

The authors declare to have no competing interests.

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