Highlights.

- STAT3 is a pro-survival, anti-apoptotic transcription factor having also a mitochondrial role
- New data indicate for STAT3 a function at the endoplasmic reticulum (ER)
- STAT3 at the ER induces degradation of the type 3 IP₃ receptor, a Ca²⁺-release channel
- STAT3 is multifaceted and also has an anti-apoptotic action via Ca²⁺ signaling modulation
- This finding indicates that transcription factors are much more pleiotropic than generally thought

**The multifaceted STAT3:
how a transcription factor regulates Ca²⁺ signaling via a degradative pathway**

Jan B. PARYS

KU Leuven, Laboratory for Molecular and Cellular Signaling,
Department of Cellular and Molecular Medicine & Leuven Kanker Instituut,
Campus Gasthuisberg O/N-1 B-802, Herestraat 49,
BE-3000 Leuven, Belgium

Jan.parys@kuleuven.be

Abstract.

STAT3 is a pleiotropic prosurvival transcription factor with functions in nucleus and mitochondria. Avalle et al. (Cell Death Diff., 2018) now present evidence that STAT3 also promotes IP₃R3 degradation at the endoplasmic reticulum, thereby limiting Ca²⁺ transfer to the mitochondria and thus supporting cellular survival via an alternate pathway.

Keywords.

STAT3

IP₃ receptor

ER-mitochondria contact sites

Oncogenes

Apoptosis

Intracellular Ca^{2+} signals are responsible for the induction and regulation of a plethora of cellular processes. For proper cell functioning, those signals have to be strictly controlled in time, space and amplitude and even slight changes in any of these parameters can lead to pathophysiological and pathological outcomes [1]. The ubiquitously expressed Ca^{2+} -release channel of the endoplasmic reticulum (ER), the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), is activated by IP_3 produced upon cell stimulation by e.g. hormones, growth factors and neurotransmitters. The activity of the IP_3R is regulated by a multitude of intracellular factors, including Ca^{2+} and ATP, various kinases and phosphatases and over 100 associated proteins [2], underscoring the enormous complexity of how Ca^{2+} signaling in general and IP_3 -induced Ca^{2+} release in particular is regulated. One of the more recent insights in that respect, was the recognition that several oncogenes and tumor suppressors perform part of their function by controlling intracellular Ca^{2+} signaling, including by direct regulation of the IP_3R [3]. This clearly demonstrates the importance of Ca^{2+} signals in cell differentiation/proliferation and in cell life/death decisions. A completely novel paradigm now emerged by the very recent finding that the ubiquitously expressed transcription factor Signal Transducer and Activator of Transcription 3 (STAT3), can indirectly modulate IP_3 -mediated Ca^{2+} signaling from the ER to the mitochondria via induction of a protein degradative process [4].

Similarly to the six other STAT family members, STAT3 is a nuclear transcription factor that after cell stimulation by in particular cytokines and growth factors. It is activated by tyrosine phosphorylation (in the case of STAT3 on Tyr^{705}), leading to its dimerization and translocation to the nucleus (Fig. 1). The main function of STAT3 is to regulate cell proliferation and survival. It therefore drives the expression of multiple genes involved in these processes, including that of anti-apoptotic genes as those coding for Bcl-2 family members. Remarkably, the latter proteins are themselves also regulators of IP_3 -mediated Ca^{2+} signaling from the ER to the mitochondria, acting on the IP_3R via several mechanisms [5].

Except for the cytosol and the nucleus, STAT3 is however also imported to the mitochondrial matrix, where it supports the electron transport chain and represses the opening of the permeability transition pore (Fig. 1). Additionally, STAT3 affects transcription of mitochondrial genes although the links between these various features as well as the underlying mechanisms are still unclear [6]. Interestingly, for most –but not all– nuclear functions, STAT3 needs to be phosphorylated on Tyr^{705} , while a phosphorylation on Ser^{727} is needed for its mitochondrial functions.

The new findings by Aavall et al. [4] now demonstrate that STAT3 has at least a third important function, now at the level of the ER. At the ER-mitochondria contact sites, STAT3 interacts with IP_3R , and especially in the region between a.a. 227 and 800 that together represents the IP_3 -binding core, and a small part of the central coupling domain. Interestingly, this association facilitates IP_3R degradation by the proteasome (Fig. 1). The degradation of a Ca^{2+} -release channel responsible for funneling Ca^{2+} to the mitochondria decreases the likelihood for the occurrence of mitochondrial Ca^{2+} overload and thus increases cellular resistance to apoptosis [7, 8].

Although the phosphorylation state of STAT3 is neither critical for its ER localization nor for its interaction with IP_3R , in keeping with its non-nuclear role, the functional effect of STAT3 on IP_3 -mediated Ca^{2+} signaling depends on its phosphorylation at Ser^{727} . The latter phosphorylation is probably needed for provoking a conformational change of the IP_3R leading to its degradation or alternatively for recruitment of a component of the degradative pathway. The importance of Ser^{727}

phosphorylation is especially relevant, as there exist two splice isoforms of STAT3, a 770 a.a. long STAT3 α that acts as an oncogene while the shorter (722 a.a.) STAT3 β rather functions as a tumor suppressor [9]. However, even if the STAT3 coiled-coil and DNA-binding domains responsible for interaction with IP₃R3 are conserved between the two STAT3 splice isoforms, the Ser⁷²⁷ critically needed for the regulation of the IP₃R3 stability is only present in the STAT3 α isoform. Therefore, the relation between STAT3 and IP₃R3 is fully in line with its oncogenic role. As other IP₃R isoforms can also participate in Ca²⁺ transfer between ER and mitochondria [10] it remains an open question whether STAT3 can also interact with and destabilize IP₃R1 or IP₃R2.

Anyway, the effect of STAT3 on IP₃R3 stability relates to other recent findings emphasizing the importance of IP₃R3 stability regulation. Similarly to STAT3, F-box protein FBXL2 that forms a subunit of a ubiquitin ligase complex interacts with the N-terminal part of IP₃R3 (in particular at a.a. 545-566 in the IP₃-binding core), promoting its ubiquitination and subsequent degradation [11]. Interestingly, BRCA-associated protein 1 (BAP1) has the opposite effect of FBXL2 as with its deubiquitinase activity it prevents IP₃R3 degradation by the proteasome after association to IP₃R3 in its N-terminal part (a.a. 1-800) [12]. It can be expected, though remains to be proven, that BAP1 can also antagonize STAT3-induced IP₃R3 degradation.

The constitutive activation of STAT3 in multiple cancer types as breast, lung, pancreatic, liver and colorectal cancers, provides the tumor cells with a distinct survival advantage, and is ultimately associated with a poor outcome for the patients [13]. STAT3 has therefore been proposed as a valid therapeutic target and several STAT3 inhibitors were developed. Most inhibitors target the STAT3 SH2 domain involved in its activation and dimerization, though their success is limited due to their incomplete inhibitory action and/or their high toxicity [14]. Inhibitors targeting other STAT3 domains are already considered, and present findings would justify investigating the possibility to act on STAT3/IP₃R3 interaction to counteract IP₃R3 degradation thereby preserving the mitochondrial apoptotic pathway.

p53 is the first transcription factor reported to modulate intracellular Ca²⁺ signals [15]. Now it appears that the transcription factor STAT3 is also acting on Ca²⁺ signaling [4]. However, its mechanism of action is completely different as p53 promotes Ca²⁺ pumping in the ER by reducing the oxidation of the ER Ca²⁺ pumps, the so-called SERCA pumps, while STAT3 promotes the degradation of IP₃R3, a Ca²⁺ channel responsible for releasing Ca²⁺ from the ER.

The newly discovered function of STAT3 at the ER underpins on the one hand the important role of intracellular Ca²⁺ handling in cell life and death decisions, especially at the ER-mitochondria contact sites, and at the other hand indicate that transcription factors can act in a much more pleiotropic and diverse way than generally thought. We certainly have to look forward to more transcription factors performing non-transcriptional functions related to intracellular Ca²⁺ handling.

Funding

Related research in the Laboratory of Molecular and Cellular Signaling was supported by grants of the Research Fund—Flanders, the Research Council of the KU Leuven, and the Interuniversity Attraction Poles Programme (Belgian Science Policy).

Conflict of interests.

None.

Figure 1. Comprehensive model displaying the main pathways by which STAT3 has an anti-apoptotic function.

The various anti-apoptotic effects of STAT3 at nuclear, ER and mitochondrial level are schematically represented. The nuclear pathway is generally initiated after cell stimulation, activation of Janus kinase (JAK) and STAT3 phosphorylation on Tyr⁷⁰⁵. The non-nuclear pathways are dependent on STAT3 phosphorylation at Ser⁷²⁷ by various kinases as mitogen-activated protein kinase (MAPK), some protein kinase C isoforms (PKCε) or the mechanistic target of rapamycin (mTOR). Activatory pathways are indicated in green and inhibitory pathways in red. STAT3-dependent degradation of IP₃R [4] will hamper IP₃-induced Ca²⁺ flux (blue arrow) from the ER to the mitochondria, thereby decreasing the possibility of mitochondrial Ca²⁺ overload and of apoptosis induction. Under normal conditions, mitochondrial Ca²⁺ supports mitochondrial bioenergetics ([ATP]) but mitochondrial Ca²⁺ overload provokes the opening of the mitochondrial permeability transition pore (mPTP) leading to mitochondrial depolarization, termination of oxidative phosphorylation, remodeling of the inner mitochondrial membrane and rupture of the outer one, and finally the release in the cytosol of pro-apoptotic factors such as cytochrome c [7]. Finally, STAT3 drives the expression of Bcl-2-family members that perform their anti-apoptotic function either by interacting with their pro-apoptotic relatives (e.g. Bax/Bak) or by controlling IP₃R activity via various mechanisms that encompass both inhibitory and stimulatory events. IP₃R inhibition by Bcl-2 is the main anti-apoptotic feature and is here depicted [5].

References

- [1] M.J. Berridge, The inositol trisphosphate/calcium signaling pathway in health and disease. *Physiol. Rev.* 96 (2016) 1261-1296. doi: 10.1152/physrev.00006.2016.
- [2] D.L. Prole, C.W. Taylor, Inositol 1,4,5-trisphosphate receptors and their protein partners as signalling hubs. *J. Physiol.* 594 (2016) 2849-2866. doi: 10.1113/JP271139.
- [3] M. Bittremieux, J.B. Parys, P. Pinton, G. Bultynck, ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca^{2+} signaling. *Biochim. Biophys. Acta.* 1863 (2016) 1364-1378. doi: 10.1016/j.bbamcr.2016.01.002.
- [4] L. Avalle, A. Camporeale, G. Morciano, N. Carocchia, E. Ghetti, V. Orecchia, D. Viavattene, C. Giorgi, P. Pinton, V. Poli, STAT3 localizes to the ER, acting as a gatekeeper for ER-mitochondrion Ca^{2+} fluxes and apoptotic responses. *Cell Death Differ.* (2018) in press. doi: 10.1038/s41418-018-0171-y.
- [5] T. Vervliet, J.B. Parys, G. Bultynck, Bcl-2 proteins and calcium signaling: complexity beneath the surface. *Oncogene* 35 (2016) 5079-5092. doi: 10.1038/onc.2016.31.
- [6] D.J. Garama, C.L. White, J.J. Balic, D.J. Gough, Mitochondrial STAT3: Powering up a potent factor. *Cytokine* 87 (2016) 20-25. doi: 10.1016/j.cyto.2016.05.019.
- [7] G. Roest, R.M. La Rovere, G. Bultynck, J.B. Parys. IP_3 receptor properties and function at membrane contact sites. *Adv. Exp. Med. Biol.* 981 (2017) 149-178. doi: 10.1007/978-3-319-55858-5_7.
- [8] G. Bultynck, M. Campanella, Tumor suppressive Ca^{2+} signaling is driven by IP_3 receptor fitness. *Cell Stress* 1 (2017) 73-78. doi: 10.15698/cst2017.11.109.
- [9] P. Aigner, V. Just, D. Stoiber, STAT3 isoforms: Alternative fates in cancer? *Cytokine* (2018) in press. doi: 10.1016/j.cyto.2018.07.014.
- [10] H. Akl, G. Monaco, R. La Rovere, K. Welkenhuyzen, S. Kiviluoto, T. Vervliet, J. Molgó, C.W. Distelhorst, L. Missiaen, K. Mikoshiba, J.B. Parys, H. De Smedt, G. Bultynck, $\text{IP}_3\text{R}2$ levels dictate the apoptotic sensitivity of diffuse large B-cell lymphoma cells to an IP_3R -derived peptide targeting the BH4 domain of Bcl-2. *Cell Death Dis.* 4 (2013) e632. doi: 10.1038/cddis.2013.140.
- [11] S. Kuchay, C. Giorgi, D. Simoneschi, J. Pagan, S. Missiroli, A. Saraf, L. Florens, M.P. Washburn, A. Collazo-Lorduy, M. Castillo-Martin, C. Cordon-Cardo, S.M. Sebt, P. Pinton, M. Pagano. PTEN counteracts FBXL2 to promote $\text{IP}_3\text{R}3$ - and Ca^{2+} -mediated apoptosis limiting tumour growth. *Nature* 546 (2017) 554-558. doi: 10.1038/nature22965.
- [12] A. Bononi, C. Giorgi, S. Patergnani, D. Larson, K. Verbruggen, M. Tanji, L. Pellegrini, V. Signorato, F. Olivetto, S. Pastorino, M. Nasu, A. Napolitano, G. Gaudino, P. Morris, G. Sakamoto, L.K. Ferris, A. Danese, A. Raimondi, C. Tacchetti, S. Kuchay, H.I. Pass, E.B. Affar, H. Yang, P. Pinton, M. Carbone, BAP1 regulates $\text{IP}_3\text{R}3$ -mediated Ca^{2+} flux to mitochondria suppressing cell transformation. *Nature* 546 (2017) 549-553. doi: 10.1038/nature22798.
- [13] X. Wang, P.J. Crowe, D. Goldstein, J.L. Yang, STAT3 inhibition, a novel approach to enhancing targeted therapy in human cancers. *Int. J. Oncol.* 41 (2012) 1181-1191. doi: 10.3892/ijo.2012.1568.

[14] J.D. Beebe, J.Y. Liu, J.T. Zhang, Two decades of research in discovery of anticancer drugs targeting STAT3, how close are we? *Pharmacol. Ther.* (2018) in press. doi: 10.1016/j.pharmthera.2018.06.006.

[15] C. Giorgi, M. Bonora, G. Sorrentino, S. Missiroli, F. Poletti, J.M. Suski, F. Galindo Ramirez, R. Rizzuto, F. Di Virgilio, E. Zito, P.P. Pandolfi, M.R. Wieckowski, F. Mammano, G. Del Sal, P. Pinton, p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+} -dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 1779-1784. doi: 10.1073/pnas.1410723112.

Conflict of interests.

None.