Cell death

https://doi.org/10.1038/s41556-024-01390-2

RIPK1 and necroptosis role in premature ageing

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Progeria, or premature ageing, is a devastating condition caused by defects in the nuclear envelope and is associated with systemic inflammation. A study now shows in animal models that inhibiting necroptosis, and particularly activity of the RIPK1 kinase, reduces inflammation and results in a meaningful extension in lifespan[1].

Premature ageing is a devastating condition with skin abnormalities, alopecia, osteoporosis, and cardiovascular problems, and results in premature death, usually before the age of 13 and mostly due to the cardiovascular issues[2]. One genetic cause of premature ageing results from a seemingly innocuous, synonymous coding mutation in the gene encoding the nuclear envelope protein lamin A. This generates an alternative splice site that leads to the in-frame deletion of 50 amino acids within prelamin A, which, while leaving the *CAAX* motif and hence farnesylation intact, removes a processing site for the metalloprotease ZMPSTE24. This results in toxic accumulation of the farnesylated, unprocessed, lamin A, known as prelamin A or progerin, on the nuclear membrane. Loss-of-function mutations in ZMPSTE24 result in similar phenotypes even though lamin A is not its only substrate[3]. This mutated protein disrupts the nuclear membrane, distorts nuclear structure, and its accumulation is the cause of the systemic inflammatory phenotype, associated with upregulation of cytokines such as IL-6 and TNF. Furthermore, blocking inflammation, genetically or with drugs, can ameliorate many aspects of the phenotype and extend life in mouse models. In this issue of *Nature Cell Biology*, Yang et al.[1] demonstrate that RIPK1, a crucial regulator of TNF signalling and cell death, and particularly its kinase function, contributes to many of the progeric phenotypes in mice[1]. Importantly, *Zmpste24*$^{-/-}$ mice on a RIPK1 kinase-dead, or, to a slightly lesser extent, necroptosis-deficient background (*Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$) have a dramatically extended survival.

The discoveries that defects in the processing of lamin A lead to systemic inflammation and progeria syndromes led to several potential therapeutic targets. Some of these have been tested pre-clinically in mice and have progressed to the clinic. In this context, it is notable how effective genetically blocking RIPK1 kinase activity, but also necroptosis, is in delaying symptoms in one mouse model, compared with other approaches already clinically translated. Thus, in a *Zmpste24*$^{-/-}$ mouse with a median survival of 17 weeks, addition of the anti-inflammatory sodium salicylate only increased survival by 4 weeks, and a heterozygous NF-κB background (*Rela*$^{-/+}$) provided approximately the same — less than 25% extension in lifespan[4]. Similarly, in a mutant *Lmna* mouse strain that can only generate progerin, median survival was roughly 20 weeks and treatment with a farnesyl transferase inhibitor (FTI) extended this to around 30 weeks[5]. Lastly, in a *Lmna* point mutant strain, which mimics the human mutation, median survival was 16 weeks and the clinical IL-6 inhibitor, tocilizumab, extended survival by just over 2 weeks[6]. Although it is not possible to precisely compare in vivo results, even when the same strains are used, owing to microbiota and other differences, it seems that the greater than twofold extension in life span (from 14 to 31 weeks) in the RIPK1 kinase-dead mutant mice is a standout of the pre-clinical studies discussed here. However, caution in interpreting the tocilizumab result is warranted because tocilizumab does not work in mice[7]. Thus, it is entirely possible that inhibiting IL-6 is a more powerful therapeutic approach than the study by Squarzoni et al.[6] indicates, and it would be interesting to test this genetically.

An exciting implication of this study from Yang et al.[1] is that small molecule inhibitors of RIPK1 kinase activity might help to treat progeria. The current US Food and Drug Administration (FDA)-approved treatment is the FTI lonafarnib, which can extend the life of patients by about 2–4 years[2,8]. As might be expected of an inhibitor of such a fundamental pathway (it has been trialled both as an anti-cancer and anti-viral drug), there are side effects associated with lonafarnib, including nausea and vomiting. These are sufficiently serious that some patients discontinue treatment[8]. On the other hand, a multitude of companies are developing specific RIPK1 kinase inhibitors and, one of the most advanced, GSK2982772, has so far demonstrated an excellent safety profile in a phase 2a clinical trial in a chronic inflammatory condition[9]. Given that genetically blocking necroptosis also provided substantial extension of life in this mouse model of progeria[1], RIPK3 or MLKL inhibitors or even dual or combined RIPK1, RIPK3 and MLKL inhibitors might also work well and be well tolerated[10,11]. The fact that genetic inhibition of RIPK1 kinase inhibition appeared to provide a greater survival advantage than genetic inhibition of necroptosis alone, and that this correlated with an effect on induction of inflammatory cytokines, may suggest a necroptosis-independent component. As RIPK1 inhibition can block TNF-induced apoptosis in certain circumstances, it is possible that it is still a TNF-induced cell death-dependent phenomenon. A challenge in studying TNF-induced inflammation in vivo and assessing the contribution of cell death is that TNF-induced cell death can be a potent inducer of TNF by neighbouring cells[11,12]. In some mouse models of TNF inflammation, in which regulation of TNF signalling itself is disrupted, inhibiting cell death has the same complete protection as inhibiting TNF, clearly suggesting that cell death is the primary driver[13,14]. In this case, the severe structural nuclear disruption in *Zmpste24*$^{-/-}$ cells presumably disrupts many other cellular responses and not just TNF-induced cell death. Nevertheless, because inhibition of necroptosis provides such a strong therapeutic effect, it will now be particularly interesting to genetically, or therapeutically, assess the effects of TNF inhibition: after all, anti-TNF drugs are tried and tested in inflammatory conditions.

In addition to these clinical implications, the study is also interesting because the presence of progerin seems to corrupt the normal cellular response to TNF[1]. TNF alone does not normally kill cells and there are only a small number of examples in which it does[12] — for example, such as where the caspase-8 cleavage site in RIPK1 is mutated[15]. ZMPSTE24-deficient cells seem to be another of these special exceptions to the general rule. Furthermore, even in situations in which TNF kills, the

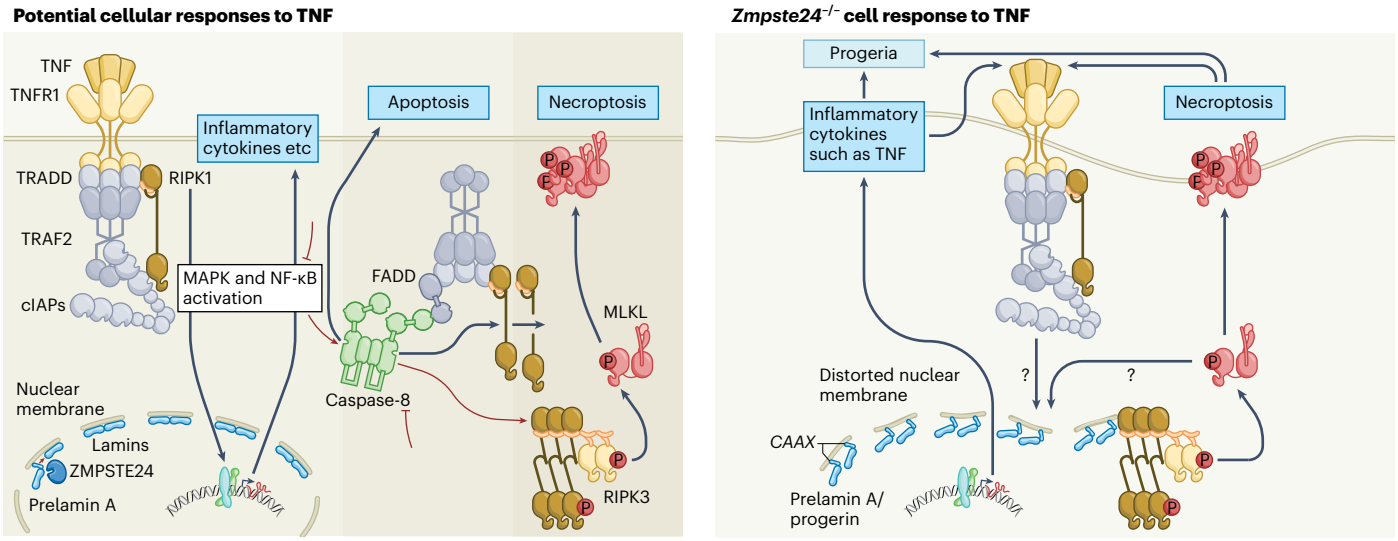

Fig. 1 | A schematic indicating potential signalling outputs from TNF/TNFR1 in wild-type and *Zmpste24*$^{-/-}$ cells. Left, the primary response to TNF is a transcriptional inflammatory response; however, if this is inhibited, it triggers activation of caspase-8 and apoptosis. If this apoptotic response is inhibited, RIPK1 and RIPK3 oligomerize, auto-activate and RIPK3 phosphorylates MLKL, leading to oligomerization and permeabilization of the plasma membrane. In *Zmpste24*$^{-/-}$ cells, the presence of unprocessed prelamin A or progerin in the nuclear membrane structurally distorts the nucleus, driving the production of inflammatory cytokines including TNF. Progerin recruits RIPK1 to the nucleus, and this results in recruitment and activation of RIPK3, bypassing or overwhelming the apoptotic cascade and directly initiating necroptosis. As necroptotic cell contents can trigger TNF production from neighbouring cells, the potential for a chronic inflammatory cycle exists.

preferred cell death pathway induced by TNF is caspase-8-dependent apoptosis. Because apoptotic cell death inhibits necroptosis, it is normally necessary to inhibit caspase-8 for TNF to activate RIPK3 and hence MLKL to cause necroptosis. Yet *Zmpste24*$^{-/-}$ cells have a pronounced predilection to respond with a necroptotic response to TNF without requiring caspase-8 inhibition. To understand this unique response, it is useful to compare it with the normal role of RIPK1 in TNF signalling. After TNF binding to TNFR1, RIPK1 is recruited to the membrane bound TNFR1 signalling complex via a homotypic death domain interaction, driving activation of MAPK and NF-κB (Fig. 1). This transcription-activating membrane-bound complex matures into a cytosolic complex that can, in the right circumstances (such as inhibition of NF-κB), recruit and activate caspase-8 via FADD to kill cells, while simultaneously inactivating the necroptotic pathway by cleaving, among other proteins, RIPK1 and RIPK3. By contrast, in *Zmpste24*$^{-/-}$ cells, although TNF is still required to activate the cell death pathway, Yang et al.[1] found that RIPK1 is recruited to the unprocessed prelamin A and this leads to activation of RIPK3, and hence activation of MLKL[1] (Fig. 1). Thus, it seems that this unconventional recruitment of RIPK1 to the nuclear prelamin A somehow side steps the involvement of FADD and caspase-8, and the lack of engagement of caspase-8, and therefore cleavage of RIPK1 may account for the fact that TNF is able to kill both RIPK1-cleavage-mutant and *Zmpste24*$^{-/-}$ cells.

Finally, there is the tantalising possibility that progeria is an accelerated version of the natural process. The idea that chronic inflammation can contribute to ageing, or 'inflammaging', is certainly plausible and the link to the current work is that there have been claims that, as we age, cells lose ZMPSTE24 expression and/or otherwise begin to express unprocessed lamin A. However, the data that support this idea, critically analysed in a recent review[3], are still underwhelming. The philosopher's stone, popularised by the first *Harry Potter* book, was believed by alchemists to be a substance that could turn base metals into gold and confer immortality. If there is anything to the idea that necroptosis contributes to natural ageing, then necroptosis inhibitors might fancifully be considered a real philosopher's stone, extending lifespan and transmutating base metals into gold for pharmaceutical companies.

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Published online: 27 March 2024

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Competing interests

J.S. is a consultant for a company developing RIPK1 inhibitors. P.W. has no competing interests to declare.