Linear ubiquitin as a common regulator of cellular stress

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Introduction

Linear ubiquitination or M1-ubiquitination (M1-Ub) is a fundamental post-translational modification that regulates cell death and inflammation in sterile conditions and in response to pathogen infection. This process is conserved in mammals and Drosophila [1]. Indeed, studies performed in Drosophila contributed to the basic understanding of the role of important regulators of inflammatory responses, such as Caspase-8, cFLIP and the E3-ligases cIAP1/2 and LUBAC. The linear ubiquitin chain assembly complex (LUBAC) is the only E3-ligase able to generate linear ubiquitin linkages identified to date. LUBAC is composed of HOIL-1, HOIP and SHARPIN. The M1-ubiquitin E3-ligase activity of LUBAC resides in HOIP, which in Drosophila is called LUBEL. M1-Ub is required for optimal activation of NF-κB and for preventing cell death downstream of a plethora of different innate and adaptive immune receptors, but it has been best characterised in the context of death receptors of the TNF receptor (TNFR) family [2].

Linear or M1-ubiquitination (Ub) is required for optimal NF-κB activation and for cell death inhibition. Using Drosophila as a model organism, Aalto et al. found that hypoxia, oxidative and mechanical stress induced M1-Ub by the HOIP homolog, LUBEL. Increased M1-Ub had a protective function driven by activation of the NF-κB transcription factor Relish via the Immune deficiency pathway (Imd). This protective M1-Ub was also induced upon cellular stress in colorectal cancer cells. Collectively, they propose that M1-Ub is a conserved, common response to different forms of stresses. These findings may have important implications for the use of HOIP inhibitors for cancer treatment.

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Abbreviations

cFLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; cIAP1/2, cellular inhibitor of apoptosis 1 and 2; HOIL-1, haem-oxidised IRP2 ubiquitin ligase 1; HOIP, HOIL-1-interacting protein; HOIPIN, HOIP inhibitor; IKKα/β, inhibitor of kappa-B kinase α and β; IL-1β, interleukin 1β; Imd, immune deficiency pathway; LUBAC, linear ubiquitin chain assembly complex; M1-Ub, M1-ubiquitination; MAPK, mitogen-activated protein kinase; NEMO, NF-kappa-B essential modulator; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OTULIN, OTU deubiquitinase with linear linkage specificity; PGRP, Peptidoglycan recognition proteins; SHARPIN, SHANK-associated RH domain interactor; TAB1/2, mitogen-activated protein kinase kinase kinase 7-interacting protein 1 and 2; TAK1, transforming growth factor-β (TGF-β)-activated kinase 1; TNF, tumour necrosis factor.
Upon TNF stimulation, LUBAC is recruited to the TNFR1 signalling complex (TNFR-SC) by K63-ubiquitin chains generated by cIAP1/2. Both K63- and M1-Ub allow for recruitment and activation of the kinases TAK1/TAB1/2 and IKKα/β/γ (NEMO) resulting in NF-κB and MAPK activation, which subsequently leads to the activation of transcription of genes involved in proliferation, survival and in the production of proinflammatory cytokines. In Drosophila, this is equivalent to the activation of the Imd pathway. However, in mammals, the TNFR-SC is also required to prevent cell death [3]. It is well established that M1-Ub regulates inflammatory gene activation and cell death upon activation of immune receptors [4]. However, little is known about the role of M1-Ub in response to other type of stresses. In the study by Aalto et al., the authors show that hypoxia, oxidative stress and mechanical stress induce M1-Ub in flies resulting in Relish (homolog for NF-κB)-mediated gene activation and protection from stress [5]. LUBEL loss or inactivation resulted in reduced survival of the flies upon these stresses in a process mediated by Imd pathway activation. Last, the authors made the interesting observation that these stresses induced M1-Ub in colorectal cancer cells and that inhibition of M1-Ub by the HOIP inhibitor, HOIPIIN, counteracted this effect and promoted stress-dependent death. Hence, inhibition of M1-Ub could be used to sensitize tumour cells to a diversity of stress responses. This concept proposes HOIP inhibition as a means to exploit tumour vulnerabilities, such as hypoxia, mechanical stress, for instance, during metastasis, or oxidative stress to kill cancer cells.

**M1-Ub regulates stress response independent of immune receptors**

Aalto et al. showed that hypoxia induced an increase in LUBEL-dependent M1-Ub [5]. However, this event was neither required for oxygen sensing nor for the activation of HIF. Instead, the authors show that this is mediated by TAK1 and Diap2, which is the cIAP1/2 homologue, and dependent on Dredd, which is the Caspase-8 homologue. In Drosophila, the Imd pathway is activated by peptidoglycan recognition proteins (PGRPs). Yet, the authors show that M1-Ub-mediated protection from stress was independent of this receptor. Although it cannot be excluded that other receptors could be involved; it is possible that increased M1-Ub upon stress induces the promiscuous recruitment of a signalling platform, equivalent to the Ripoptosome [6] that could eventually be assembled in a receptor-independent manner. Alternatively, the Imd complex could be assembled during stress response and induce LUBEL activity within the complex. The authors provide alternative plausible hypotheses as to what could be the potential targets or pathways mediating this effect, spanning from recognition of changes in intracellular patterns, induced during hypoxia, by Toll receptors, to binding of HOIP to previously ubiquitinated proteins resembling the events reported during Salmonella infection [7].

The understanding of the role of M1-Ub in platforms that are not associated with death receptor complexes is particularly interesting in the context of mutations that cause increased M1-Ub. The best example would be mutations in the LUBAC-specific deubiquitinase, OTULIN. Catalytically inactive or complete loss of OTULIN results in massively increased M1-Ub and embryonic lethality [8–10]. The latter resembles HOIP deficiency, which in contrast to OTULIN mutations, results in complete loss of M1-Ub. There is much controversy around the reason behind this dichotomy. Whilst it is not clear for OTULIN absence, OTULIN inactivation impairs recruitment of LUBAC to, and M1-Ub at, the TNFR-SC, despite presenting increased M1-Ub in overall cellular lysates [8,11]. Indeed, catalytically inactive OTULIN mutant mice perish due to exacerbated cell death, just like the phenotype of LUBAC-deficient mice [8]. On the contrary, OTULIN deficiency was reported to cause excessive NF-κB activation and is associated with chronic inflammation in humans (ORAS syndrome) [10]. There is, unfortunately, no evidence of a functional death receptor signalling complex in the context of OTULIN deficiency. Yet, based on the findings presented by Aalto et al. [5], it may be possible that M1-Ub aberrantly generated by the loss of OTULIN and that is not associated with death receptors is inducing the promiscuous activation of signalling platforms that cause a constitutively activated NF-κB mediated inflammatory response. Both the differences and similarities between loss of HOIP, OTULIN or its catalytic inactivation are puzzling, and it will be extremely interesting to see what the future research will teach us about how linear ubiquitination regulates immune and stress responses at the level of immune receptor complexes and/or cytoplasmic signalling platforms.

**M1-Ub in hypoxia associated with obesity**

The finding that M1-Ub is specifically required for activation of Relish (NF-κB) in the fat body in flies during hypoxia and oxidative stress is very intriguing.
Obesity is a chronic hypoxic state, which causes adipose tissue dysfunction, inflammation and insulin resistance [12]. These events eventually cause metabolic disorders including cardiovascular disease and diabetes. Other than hypoxia, oxidative stress contributes to the metabolic changes occurring during obesity [12]. In addition, it is well known that obesity induces the production of cytokines, such as TNF and IL-1β, which activate immune responses [13]. Hence, the findings described by Aalto et al. [5] may have important implications for understanding the mechanisms behind obesity-associated inflammation. Could it be possible that M1-Ub is implicated in fine-tuning stress response and immune signalling during adipose tissue expansion?

**M1-Ub in cancer**

The authors observed increased M1-Ub levels in response to hypoxia and other stresses in CaCo2 cells and the inhibition of HOIP, using HOIPINs, prevented this [5]. However, no changes were observed during hypoxia or mechanical stress at the level of NF-κB activation in these cells. It should be noted that in many different scenarios we and others observed that loss of M1-Ub results in exacerbated cell death but it does not always result in impaired NF-κB activation. In Drosophila, the authors show that the protective effect of LUBEL during stress response was dependent on Caspase-8. Although it is difficult to study cell death responses in Drosophila, this finding implies that in mammals LUBAC prevents the formation of a cell death platform upon stress response and, thus, the protection afforded by LUBAC activity might not be linked to NF-κB activation in this context. Indeed, the authors show that inhibition of M1-Ub by HOIPINs during hypoxia sensitised CaCo2 cells to death. Another report showed that even though hypoxia upregulated NF-κB responses in line with Aalto et al. [5], this was not dependent on HOIP or HOIL-1 [14]. However, as discussed above, LUBAC-mediated regulation of stress response may be more prominent at the level of cell death inhibition rather than NF-κB activation.

The study of the role of M1-Ub in tumourigenesis is quite young. Here, the authors propose that M1-Ub could be used as a target to trigger death under hypoxic conditions in colorectal cancer. Other studies support this concept. For instance, HOIP overexpression promoted lymphomagenesis [15] and OTULIN deficiency in hepatocytes promoted liver cancer [16]. Both models support the protumorigenic functions of increased and/or sustained M1-Ub. On the contrary, HOIP deficiency in hepatocytes promoted hepatocarcinoma which could be caused by sustained low-grade cell death levels and inflammation during early life [17]. The phenotypes of OTULIN and HOIP deficiencies on liver cancer remain, yet again, to be fully understood. As discussed in the previous section, whether the study by Aalto et al. [5] can provide hints on what the underlying differences between OTULIN and HOIP deficiencies are during tumourigenesis remains to be addressed.

**Conclusions**

Hypoxia and oxidative stress are major processes that affect chronic inflammatory diseases such as obesity and cancer. The study by Aalto et al. [5] proposes M1-Ub as a common regulator of stress response in Drosophila and, potentially also, in mammals. Although the implication of M1-Ub during stress response in mammals requires further investigation, it certainly holds promise. Most importantly, the authors provide evidence in favour of exploiting therapies based on LUBAC modulation to boost cell death upon stress responses in colorectal cancer. Hence, it will be exciting to see the developments of LUBAC inhibitors as a therapeutic strategy for cancer treatment but also for the treatment of other degenerative diseases.

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**Conflict of interest**

The author declares no conflict of interest.

**Author contributions**

NP wrote the manuscript.

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