Details Unfold: The ER Stress Response in Intestinal Inflammation and Cancer

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Following intestinal injury and epithelial cell loss, rapid regeneration of the epithelium is essential to maintain barrier integrity and host defence. However, an unrestrained proliferative response to injury may promote the development of intestinal tumours. Accumulating evidence reveals links between intestinal inflammation, tumourigenesis, and defects in cellular stress response mechanisms such as the endoplasmic reticulum (ER) stress response. Accumulation of aberrant proteins in the ER resulting from inflammation, infection, injury or high protein turnover triggers a set of stress response signalling pathways collectively termed the unfolded protein response (UPR). Activation of UPR pathways leads to upregulation of various mechanisms to restore cellular homeostasis or induce apoptosis if stress remains unresolved. An effective UPR is therefore particularly important for maintaining homeostasis in the intestinal epithelium, which is a site of frequent inflammation and injury and which contains cells with high protein turnover such as secretory goblet cells and Paneth cells. Genetic variants of UPR pathway components are known to confer risk to inflammatory bowel disease, including colitis-associated cancer (CAC) and colorectal cancer (CRC). In the paper here discussed, Niederreiter and colleagues explore the mechanism for this association, finding that the transcription factor X-box binding protein 1 (Xbp1), an effector of the UPR, acts to regulate local inflammation during ER-stress and restricts proliferative and regenerative responses to inhibit intestinal tumour development and progression (J Exp Med. 2013 210(10):2041-56).

Under conditions of ER-stress, the transcription factor Xbp1 initiates cytoprotective responses including upregulation of protein chaperones and enhanced ER-associated protein degradation (ERAD) to restore cellular homeostasis. To explore the specific role of the UPR in intestinal epithelial cells (IEC), Kaser and colleagues had previously generated a transgenic mouse model mimicking ER-stress in intestinal epithelia by targeted deletion of Xbp1. Xbp1 knockout in mouse IECs disrupts the UPR and results in spontaneous enteritis and increased susceptibility to induced colitis (Cell 2008, 134:743-756).

In the present paper, the authors found that loss of Xbp1 in epithelial cells also caused an increase in number of both crypt base intestinal stem cells (ISC) and transit-amplifying (TA) cells, in accordance with previous observations of high epithelial turnover in this model (Cell 2008, 134:743-756). The authors then generated a transgenic model in which both Xbp1 and the ER-stress sensor inositol-requiring enzyme 1α (Ire1α) were deleted. Ire1α detects unfolded or misfolded proteins in the ER and initiates a UPR response via activation of Xbp1. Using these double-knockout mice they were able to show that under conditions of unresolved ER-stress (Xbp1 deficiency) activation of the stress-sensor Ire1α was central to proliferation in the crypt base ISCs, but had no observed effect on homeostatic ISC function in the absence of ER-stress.

In addition to effects on crypt base ISCs, Xbp1 deficiency also causes Paneth cell dysfunction (Cell 2008, 134:743-756). Paneth cells reside alongside ISCs in the crypt base where they perform a protective role in releasing antimicrobial peptides and inflammatory mediators (Nat Rev Microbiol 2011, 9:356-68) and are also contribute to maintenance of the ISC niche (Nature 2011, 460:415-8; PNAS 2012, 109: 8965-70; PNAS 2012, 109:3932-7; Nature 486:490-5). To examine whether ISC expansion during ER-stress is dependent on functional adjacent Paneth cells, the authors exploited the mosaicism of the Xbp1 knockout model. In the vast majority of crypts, Xbp1 deficiency results in damaged Paneth cells (which lose lysozyme staining and lack typical morphology), however, around 5% of crypts retain apparently unaffected Paneth cells (J Exp Med. 2013 210(10):2041-56). Stem cell numbers were compared in crypts with intact or damaged Paneth cells. Only crypts with damaged Paneth cells exhibited ISC expansion in response to Xbp1 deficiency, demonstrating that hyperproliferation of ISCs under ER-stress is not a consequence of a local inflammatory environment, but is dependent on Paneth cell dysfunction, secondary to ER-stress. Local mRNA expression analysis also identified increased levels of Paneth cell-specific Wnt11 (Wnts are potent promoters of stem cell proliferation) suggesting ISC expansion in ER-stressed epithelium could be influenced by increased local Wnt release from damaged Paneth cells.

Unlike the crypt base ISCs, proliferation in the transit-amplifying (TA) epithelial cells further up the crypt was found to be independent of Ire1α, suggesting a different mechanism for the increased turnover observed in these cells. To delve deeper into the molecular events connecting ER-stress to hyperproliferation in the TA cells, the authors assessed the potential involvement of signalling via JAK1/STAT3. JAK/STAT systems are major signalling pathways involved in regulating cell proliferation, survival, migration, signalling, inflammation and tumourigenesis (Semin Immunol 2014 S1044-5323(13):00161-9; *JAK-STAT* 2013 2(4):e25530). Activation of cell surface receptors by immune mediators such as interferons, growth factors and cytokines, leads to the activation STAT heterodimers which translocate to the cell nucleus to activate a wide range of effector genes. In ER-stressed epithelium, the authors noted a substantial increase in both total and activated JAK1 and STAT3 in TA epithelial cells, but not in the crypt base ISCs. Using the Xbp1/Ire1α double knockout model, they went on to show that hyperproliferation of TA cells in ER-stressed epithelium is mediated via the STAT3 pathway.

To determine whether STAT3 activation results from cell-intrinsic mechanisms or via signalling from the local environment, the authors examined JAK1/STAT3 activation in an intestinal epithelial cell line. In accordance with the *in vivo* data, inducing ER-stress in this cell line again resulted in a substantial increase in total and activated JAK1 and STAT3, confirming that STAT3 activation in response to ER-stress can be an IEC-intrinsic process. Using specific neutralising antibodies, the authors were then able to show that cytokines IL-6 and IL-11 released from the IECs themselves could signal in an autocrine manner to activate STAT3 via the NF-κB pathway, which connects the ER-stress response with immune signalling.

Finally, the authors investigated the roles of Xbp1 in the development and progression of intestinal tumours, using a chemically induced mouse model of colorectal cancer (CRC) and a model of colitis-associated cancer (CAC). Increases in tumour size and number were observed in Xbp1 knockouts compared to wild-type mice. They went on to show that the tumour-suppressive protective effect of Xbp1 is mediated through the IRE1α ER-stress response pathway, as co-deletion of the ER-stress sensor IRE1α eliminated the tumourigenic effect caused by Xbp1 deficiency. In summary, the authors demonstrate an important role for Xbp1 in limiting the regenerative, proliferative response to epithelial stress in intestinal stem cells, thereby protecting against the development and progression of intestinal tumours.

**Comment**

Intestinal stem cell activity is regulated by a complex array of signals from stromal cells and neighbouring epithelial cells which balance cell proliferation and differentiation to maintain epithelial homeostasis. Principal among these is Wnt signalling, which promotes stem cell proliferation and pluripotency (reviewed in Cell 2013, 154:274-282; Cell Sig 2014, 26:570-9). Disruption of Wnt pathways is associated with crypt loss, while over-activation can induce hyperproliferation of ISCs and tumourigenesis. Although the central importance of Wnt signalling in somatic stem cell maintenance is well characterised, additional regulation by ER-stress response pathways is less well understood.

By disrupting the UPR to induce ER-stress in epithelium, Niederreiter *et al.* find an increase in intestinal stem cell (ISC) numbers and a concomitant increase in Paneth cell-specific Wnt signalling. ISC expansion only occurred when ER-stress sensing by Ire1α was intact, and solely in crypts with damaged Paneth cells. However, it was not possible to dissect whether the lack of ISC expansion in some crypts is due to the remaining presence of normally functioning Paneth cells, or merely reflects the absence of unresolved ER-stress in the stem cells themselves (e.g. due to incomplete efficiency of the Xbp1 knockout model or localised stress resistance). Paneth cells are not necessarily required for ISC expansion, as studies in other mouse models report that Paneth cell ablation does not prevent homeostatic stem cell renewal (PNAS 2012, 109:3932-7). Furthermore, other local cell types such as sub-epithelial myofibroblasts can provide alternative sources of Wnts and additional proliferation signals (PNAS 2007, 104:15418-23; PLoS One 2014, 9:1:e84651). The degree to which signalling from Paneth cells and/or other niche cells is required for ISC expansion under conditions of ER-stress therefore remains to be determined.

Downstream of IRE1α, various adaptor proteins and transcription factors also link the ER-stress response to inflammatory signalling (Science 2000, 287:664-6; Science 2006 312:572-6; Physiol Rev 2011, 91:1219-1243; Tends Biochem Sci 2011, 36:329-337) and there is significant homology between UPR sensors proteins and immune receptors. An inflammatory environment resulting in stress-induced cytokine release from epithelial cells, or local immune cells of the underlying lamina propria, can promote mucosal pathology (Nature 2013 503:272-9) and increase the likelihood of developing intestinal cancers (Semin Immunopathol 2013, 35:307-319). Links between the UPR and intestinal inflammatory disease were initially described in studies of mice lacking Ire1β, which is specifically expressed in intestinal epithelium (J Clin Invest 2001, 107:585-593). Ire1β deficiency led to ER-stress in epithelial cells, and increased susceptibility to chemically-induced colitis (J Clin Invest 107:585-93). Subsequent studies described further associations of unresolved ER-stress with inflammatory bowel disease (IBD), Crohn’s disease (CD) and ulcerative colitis (UC) (Cell 2008 134:743-756). Pro-inflammatory stimuli such as lipopolysaccharide (LPS) which engage Toll-like receptors (TLRs) expressed on epithelial cells and/or lamina propria cells can specifically trigger XBP1 activation to enhance the transcription of pro-inflammatory cytokines, such as IL-6 (Nature Immunol 2010 11:411–418). In this paper, Niederreiter and colleagues highlight the importance of autocrine IL-6 signalling by IECs in driving epithelial hyperproliferation to promote tumourigenesis (J Exp Med 2013, 210:2041-56).

Immune receptor signalling via cytokines, interferons and growth factors is mediated by JAK/STAT pathways (Science 2002, 296:1653-5) which are implicated in several models of intestinal cancer (Nat Rev Cancer 2009, 9:798-809).There is extensive overlap between inflammatory signalling, the STAT3 pathway and the Ire1α branch of the UPR, providing opportunities for a mechanistic link between Ire1α activation and tumourigenesis. Studies in a mouse model of colitis-associated cancer (CAC) highlighted the importance of STAT3 signalling in promoting epithelial proliferation and survival (Cancer Cell 2009, 15:103-13). Inflammatory cytokine activation of STAT3 in IECs was also shown to promote tumour progression in the Apcmin mouse model of colorectal cancer (CRC) (Immunity 2004, 21:491-501), while STAT3 knockout in the IECs of these mice resulted in reduced inflammation and protection from CRC (Cancer Cell 2009, 15:91-102). Niederreiter and colleagues show here that sustained ER-stress response is tumourigenic in both CRC and inflammatory CAC models. Tumourigenesis was dependent on ER-stress (IRE1α activation) and STAT3-dependent hyperproliferation of transit-amplifying IECs (J Exp Med 2013, 210:2041-56).The authors went on to show that show that the downstream effector of Ire1α, Xbp1, restricts hyperproliferation in the TA compartment by limiting STAT3 activation. Xbp1 is therefore important in executing the ER-stress response, while restricting over-activation to protect against tumour development.

There are three main branches of the UPR, initiated by the sensor proteins Ire1α, PKR-like ER-kinase (PERK) or activating transcription factor-6 (ATF6). Activation of these pathways has been implicated in both pro- and anti-oncogenic outcomes (Nat Rev Drug Dis 2013, 12:703-19). ER-stress sensing by both the Ire1α and PERK pathways has been implicated in promoting cell transformation and tumour progression, while the ATF6 pathway has a proposed role in tumour cell dormancy (PNAS 2008, 105:10519-24). It remains unclear precisely which functions of the UPR are most important in oncogenesis, but Niederreiter and colleagues here make a significant contribution by showing that during unresolved ER-stress, the STAT3-mediated proliferative effects of Ire1α activation are central to tumour development and progression in intestinal epithelium.

Much remains to be learnt about the details of UPR both in terms of maintaining cellular homeostasis and its involvement in disease. The degree to which the different branches of the UPR are important in different cell and tissue types is incompletely understood. Furthermore, it is not known how the UPR integrates diverse inputs relaying information on the duration and intensity of ER stress with local environmental factors and metabolic status to promote cell adaptation or death. These are important questions, as a growing body of work links the UPR not just with intestinal disease but also with a range of neurodegenerative disorders such as Parkinson’s and Huntington’s, inflammatory diseases including rheumatoid arthritis, metabolic diseases including diabetes, and many cancers.

Components of the UPR therefore make compelling targets for pharmacological intervention. Screening studies have identified various compounds that can ameliorate a range of UPR-related diseases. These compounds fall into two main classes: those that inhibit the pro-survival effects of the UPR to limit tumour development and progression, and those that promote cellular adaptation to stress (Nat Rev Drug Dis 2013, 12:703-19). Those inhibiting pro-survival effects include inhibitors of Ire1α and PERK, modulators of protein degradation (e.g. ERAD), and modulators of chaperones and other quality control proteins. Those that modulate Ire1α activity can do so by inhibiting its RNase function, or by promoting Ire1α oligomerisation to increase activation. For example, compounds which block Ire1α splicing of Xbp1 can be used in *in vitro* and *in vivo* models to treat multiple myeloma (PNAS 2003, 100:9946-51; Blood 2011, 119:5772-81, Blood 2011 117:1211-14).

Targeted gene therapy, in which recombinant viruses are used to deliver active UPR components to specific tissues, has shown promise for use in a range of central nervous system disorders linked to the UPR. Targeted expression of Xbp1s using this system can reduce neuron loss in a mouse model of induced Parkinson’s (Brain Res 2009, 1257:16-24) and improve locomotor recovery after spinal injury (Cell Death Dis 2012, 3:e272) however this method is yet to be exploited in the treatment of tumours. Whether delivering Xbp1s to the intestinal epithelia could prevent or reduce tumour development in inflammatory diseases of the intestine is unknown. The tumour-suppressive role of Xbp1s in intestinal epithelial cells described here by Niederreiter *et al*, combined with the promising findings of gene therapy trials targeting the UPR in neurodegenerative disorders suggests that this approach may be worth investigating for the treatment of intestinal disease.