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Tumor Stress Inside Out: Cell-Extrinsic Effects of the Unfolded Protein Response in Tumor Cells Modulate the Immunological Landscape of the Tumor Microenvironment

Navin R. Mahadevan and Maurizio Zanetti

The unfolded protein response (UPR) is a eukaryotic cellular adaptive mechanism that functions to cope with stress of the endoplasmic reticulum (ER). Accumulating evidence demonstrates that the tumor microenvironment contains stressors that elicit a UPR, which has been demonstrated to be a cell-intrinsic mechanism crucial for tumorigenesis. In addition, the UPR is a source of proinflammatory signaling whose downstream mediators may hamper antitumor immunity. We discuss how the UPR may impair Ag presentation, which could result in defective T cell priming, also leading to tumor escape and growth. Further, we discuss the recent finding that ER stress and attendant proinflammation can be transmitted from ER-stressed tumor cells to myeloid cells. The ideas presented suggest that, in addition to being a cell-intrinsic mechanism of tumor survival, the tumor UPR can serve as a cell-extrinsic regulator of tumorigenesis by remodeling the immune response in the tumor microenvironment. *The Journal of Immunology*, 2011, 187: 4403–4409.

As early as the mid-1800s, histological examination of neoplastic tissues revealed infiltration by inflammatory cells, suggesting a close association between tumor development and inflammatory processes (1). Recent studies added molecular detail to these early observations, highlighting the role of tumor- and leukocyte-derived inflammation in promoting tumor growth. Master regulators of inflammation, such as NF- κ B and STAT3, were shown to be crucial for tumor growth, and the tumorigenic roles of some of their downstream effectors, including cytokines, chemokines, and growth factors, have been elucidated. It is now appreciated that the immune landscape of the tumor microenvironment undergoes continuous dynamic remodeling, and successful tumor outgrowth is contingent upon subversion and escape from cell-extrinsic immune control (2). In addition

to directly facilitating tumor growth, inflammatory signals in the tumor microenvironment can subvert the local immune response against cancer cells by inhibiting APC and CD8 T cell function, promoting regulatory T cell (Treg) differentiation, and hampering the development of T cell memory (3, 4). Although much work has focused on the genetic underpinnings of tumor-derived inflammation, less consideration has been given to microenvironmental and metabolic factors that could initiate and sustain inflammation in the tumor microenvironment. Recent evidence suggests that pathophysiologic conditions unique to the tumor microenvironment initiate tumor cell stress signals that converge upon the endoplasmic reticulum (ER), resulting in a condition termed “ER stress” (5). In this article, we discuss the ER stress response, its determinants in the tumor microenvironment, its newly appreciated role in tumorigenesis, and new links between tumor cell ER stress and immune cells.

ER stress and the unfolded protein response

The ER is the initial checkpoint for the biosynthesis, folding, assembly, and modification of membrane-bound and secreted proteins in eukaryotic cells. Increase in physiological demand for protein folding or stimuli that disrupt the ability of proteins to fold cause the accumulation of un-/misfolded proteins in the ER lumen, resulting in a condition known as ER stress. This activates intracellular signaling pathways known collectively as the unfolded protein response (UPR), which facilitates cellular adaptation to ER stress (6). In mammalian cells, the UPR is initiated by three ER membrane-bound sensors, IRE1 α , ATF6, and PERK, which, in unstressed cells, are maintained in an inactive state through luminal association with the ER chaperone molecule GRP78 (7). When a cell experiences ER stress, GRP78 disassociates from each of the three sensor molecules to preferentially bind un-/misfolded proteins, allowing each sensor to activate downstream-signaling cascades, which act to normalize protein folding and secretion (8).

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Abbreviations used in this article: ER, endoplasmic reticulum; IKK, I κ B kinase; MDSC, myeloid-derived suppressor cell; ROS, reactive oxygen species; TAM, tumor-associated macrophage; TERS, transmissible endoplasmic reticulum stress; TIDC, tumor-infiltrating dendritic cell; TRAF2, TNF- α R-associated factor 2; Treg, regulatory T cell; UPR, unfolded protein response.

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Upon disassociation from GRP78, IRE1 α autophosphorylates, thereby activating its endonuclease function to remove a 26-bp-segment from the *Xbp-1* mRNA transcript. Translation of the shortened transcript yields an XBP-1 isoform (XBP-1s) (9), which acts as a powerful driver of the production of various ER chaperone proteins that function to restore ER homeostasis by promoting proper protein folding, maturation, secretion, and degradation. XBP-1s also promotes the transcription of *GRP78*, *XBP-1*, thus establishing a positive feedback loop (10). Activated ATF6 translocates to the Golgi where it is cleaved into its functional form, which acts in parallel with XBP-1s to restore ER homeostasis (11). Upon release from GRP78, PERK phosphorylates eIF2 α , resulting in the selective inhibition of translation (12), effectively reducing ER client protein load. If ER stress persists, downstream signaling from PERK via ATF4 can also activate the transcription factor, CHOP, which can initiate apoptosis (13). The UPR intersects extensively with cell death signaling and can upregulate caspases, proapoptotic transcription factors, and mitochondrial death pathways, as well as inhibit antiapoptotic transcription factors (reviewed in Ref. 14).

The tumor microenvironment harbors noxae that elicit the UPR in tumor cells

The tumor microenvironment differs markedly from that of normal tissues. Most notably, tumors lack a well-developed blood supply, which, coupled with the rapid proliferation of tumor cells, leads to hypoxia, decreased glucose and amino acid supply, and low extracellular pH (5). Compounding these extrinsic noxae are tumor-intrinsic stressors, such as errors in the biosynthesis of glycoproteins and lipids (15, 16), and viruses (17), which collectively induce ER stress.

Hypoxia

Several lines of evidence indicate that hypoxia activates the UPR in tumor cells. Microarray analysis of *ras* and *c-myc* transformed mouse fibroblasts exposed to severe hypoxia in vitro revealed that multiple elements of the UPR, including *Chop*, *Grp78*, *Xbp-1*, and *Atf4*, are significantly upregulated compared with normoxic cells. Human fibrosarcoma and lung carcinoma cells upregulate GRP78 and increase XBP-1 splicing under hypoxic conditions in vitro (18, 19). Transgenic mice with spontaneous mammary carcinogenesis, in which *Xbp-1* splicing is marked by luciferase expression, showed that XBP-1s bioluminescence correlates strongly with increasing tumor hypoxia and colocalizes with CA-9, a marker of hypoxia. In human colon cancer cells, hypoxia induces PERK-dependent eIF2 α phosphorylation and ATF4 production (20).

Glucose deprivation

A high rate of tumor cell glycolysis and poor vascularization combine to severely limit the glucose available to tumor cells (21, 22). It has long been known that glucose deprivation activates cellular stress responses. The GRP protein family, including GRP78, was originally discovered because of its upregulation in response to glucose deprivation (23). In their in vivo model of microenvironment-driven tumor cell ER stress, Spiotto et al. (24) demonstrated that XBP-1-luciferase activity in spontaneous mammary tumors significantly increases when tumor-bearing mice are treated with 2-deoxy-

glucose (a nonmetabolizable glucose analog) and that glucose deprivation of tumor cells ex vivo causes a dramatic increase in XBP-1-luciferase expression. Taken together, these findings demonstrate that hypoxia and low glucose are physiologically relevant inducers of ER stress in tumors in vivo.

The UPR is activated in tumors and is essential for tumorigenesis

Accumulating evidence demonstrates UPR activation in tumor cells and its critical role in solid tumor growth and progression. Primary human tumor cells of several origins, including breast (25), lung (26), liver (27), colon (28), prostate (29), and brain (30), were shown to upregulate UPR pathways, including GRP78, ATF6, and XBP-1 splicing, whereas peritumoral areas do not. Additionally, in primary human melanoma, liver, and breast cancer specimens, the level of GRP78 positively correlates with tumor progression (25, 27, 31).

The functional link between the UPR and tumorigenesis was initially suggested by the finding that silencing of *Grp78* in mouse fibrosarcoma cells inhibited growth in an in vivo syngeneic transplantation model due, in part, to increased tumor cell-specific memory T cell generation (32). The essential role of GRP78 in tumorigenesis was confirmed when it was shown that *Grp78* heterozygous mice crossed with *MMTV-PyT* heterozygous transgenic mice display significantly decreased breast tumor proliferation, survival, and angiogenesis compared with *Grp78*^{+/+}, *PyT* mice (33). Similarly, the conditional homozygous knockout of *Grp78* in the prostates of mice with *Pten* inactivation protects against prostate cancer growth (34).

More recent work demonstrated that UPR signaling downstream of Grp78 facilitates tumor growth. Transformed mouse fibroblasts deficient in *Xbp-1* are more sensitive to hypoxic stress in vitro than wild-type cells and do not grow as tumors when injected into SCID mice. Consistent with these findings, fibroblasts expressing a small interfering RNA against *Xbp-1* led to tumors that are smaller and exhibit decreased angiogenesis compared with tumors generated by control cells when injected into mice (19, 35). Similarly, small interfering RNA inhibition of *XBP-1* in human fibrosarcoma cells inhibits their growth and angiogenesis in a xenograft model, and overexpression of XBP-1s in human fibrosarcoma cells expressing a dominant-negative IRE1 α mutant rescues xenograft angiogenesis (19, 35). Additionally, human glioma cells expressing a dominant-negative IRE1 α mutant display a decreased growth rate and impaired angiogenesis when orthotopically transplanted into immunodeficient mice (18). PERK signaling also supports tumor growth during conditions of ER stress. The inactivation of ER stress signaling by mutations of PERK, or by the introduction of a dominant-negative PERK, in mouse fibroblasts and human colon cancer cells results in tumors that are smaller, grow less rapidly, and display impaired angiogenic ability when grafted into immunodeficient mice (20, 36). Taken together, these results underscore the key contribution of the UPR in the growth and progression of solid tumors of diverse origins.

The UPR intersects with cellular proinflammation

In addition to coping with an increased un-/misfolded protein load in the ER, the UPR activates a proinflammatory cascade with tumor-promoting and cell-survival properties. NF- κ B is one of the key inflammatory regulators inducible by the UPR

(37). Each of the three UPR-signaling pathways activates NF- κ B translocation to the nucleus via distinct mechanisms and consequent inflammatory gene transcription. PERK leads to NF- κ B activation via a unique mechanism in which translational inhibition reduces the ratio of I κ B/NF- κ B, thus permitting the nuclear migration of NF- κ B and transcription of downstream inflammatory genes (38, 39). Upon autophosphorylation, IRE1 α forms a complex with TNF- α R-associated factor 2 (TRAF2) at its cytosolic domain, and the IRE1 α -TRAF2 complex mediates direct I κ B phosphorylation via I κ B kinase (IKK), which leads to NF- κ B activation. IRE1 α -deficient mouse fibroblasts under ER stress display reduced NF- κ B activation and downstream TNF- α production (40). Most recently, ATF6 was shown to participate in NF- κ B activation in an AKT-dependent manner (41). That the UPR activates NF- κ B via three independent mechanisms suggests that the ER stress response is a key regulator of cellular inflammation.

The UPR can also activate the JNK-AP-1 pathway of inflammation. The IRE1 α -TRAF2 complex, aided by ASK and AIP (42), can recruit JNK, which phosphorylates the transcription factor, AP-1, leading to inflammatory gene expression. Supporting this model, IRE1 α - or AIP-deficient fibroblasts under ER stress fail to activate JNK and downstream inflammatory responses (42, 43).

Cross-talk between the UPR and inflammatory pathways is not unidirectional. Inflammatory cytokines and reactive cell metabolites can themselves induce the UPR. For instance, TNF- α activates IRE1 α , ATF6, and PERK in mouse fibrosarcoma cells by inducing the accumulation of reactive oxygen species (ROS) (44). Additionally, IL-6, IL-1 β , and LPS activate the UPR in the liver *in vivo* (45). Furthermore, the UPR can cause the accumulation of, and be induced by, ROS, which were shown to induce phosphorylation of I κ B in T cell lymphoma and cervical cancer cells, permitting NF- κ B-dependent gene transcription (46). Although the UPR has antioxidant function via PERK/ATF4/NRF2 signaling (47), prolonged ER stress can cause the accumulation of ROS via several mechanisms: accumulation of misfolded proteins causes Ca²⁺ leak from the ER that stimulates mitochondrial ROS production, which itself can increase ER Ca²⁺ depletion; depletion of ATP to fuel protein folding induces mitochondrial respiration, whose byproducts include ROS; and formation of ROS as the consequence of formation and breakage of disulfide bond and during normal protein folding (48). Notably, limiting ROS accumulation *in vivo* using an antioxidant attenuates ER stress (49), indicating that ROS themselves also elicit the UPR.

The phenotype and immunosuppressive effects of tumor-infiltrating myeloid cells

Tumor-infiltrating myeloid cells can account for up to 30% of leukocytes in tumors (50) and are key players in the cell-extrinsic regulation of tumor growth. Because of their crucial role in priming adaptive immunity, their subversion by tumor cells represents one of the key mechanisms by which tumors escape immune control. The tumor-infiltrating myeloid population was initially characterized as heterogeneous, comprising CD11b⁺/Gr1⁺ myeloid-derived suppressor cells (MDSC), which contain, and may be precursors to (51), subpopulations of CD11b⁺/Gr1⁺/F480⁺ tumor-associated

macrophages (TAM) and CD11b⁺/CD11c⁺ tumor-infiltrating dendritic cells (TIDC) (52). Because of their ability to inhibit T cell responses *in vitro* and *in vivo* (53, 54), and the initial characterization of their phenotype as IL-10⁺/IL-12⁻, coupled with low levels of costimulatory molecules and Ag-presentation machinery, it was proposed that tumor-associated CD11b⁺/Gr1⁺ myeloid cells possessed an anti-inflammatory and suppressive phenotype (50). TIDC were first identified as having an immature phenotype characterized by low levels of MHC class I and II, as well as costimulatory molecule (CD86/CD80) expression, which was assumed to be responsible for the dysfunctional T cell priming and induction of anergy observed in these cells (55).

Evidence has accumulated that implicates tumor- and host-derived inflammatory processes in the accumulation and activity of MDSC (reviewed in Ref. 56). The inflammation-associated, proangiogenic factor vascular endothelial growth factor stimulates the generation of MDSC in a paracrine and autocrine manner (57, 58). Tumor- and host-derived IL-1 β and IL-6 cause increased MDSC burden, and increased MDSC suppressive activity and tumor outgrowth are associated with tumor-derived IL-1 β (59, 60). Cyclooxygenase-2-derived PGE₂ secreted by tumor cells polarize myeloid cells to a suppressive phenotype (61, 62). Collectively, these data may also explain recent findings that myeloid cells within the tumor microenvironment can have an inflammatory/activated phenotype while concurrently inhibiting antitumor immune responses and aiding tumor growth. For instance, in tumor-associated myeloid cells, generation of ROS crucial for the inhibition of T cell responses can occur via arginase, a classical M2 marker, but also via inducible NO synthase, an inflammatory marker (53, 63). Furthermore, as discussed below, tumor-derived myeloid cells produce inflammatory cytokines, such as IL-6, IL-23, and TNF- α , which play key roles in tumor growth and in regulating antitumor immunity (3). More recently, it was found that TIDC in melanoma (64), lung carcinoma (65), and breast cancer (66) express high levels of MHC class I/II, CD80, and CD86, yet they still inhibit antitumor CD8 T cell responses *in vitro* and *in vivo* as a result of inadequate Ag presentation and arginase production (65–67). Taken together, these results underscore a heretofore unappreciated phenotype of tumor-associated myeloid cells as both inflammatory and suppressive (68). The tumor-derived signals driving this mixed inflammatory/suppressive phenotype have yet to be elucidated.

Although only a few studies have investigated Ag processing and presentation in tumor-associated myeloid cells, studies have shown that inhibition of TCR engagement of specific peptide/MHC complexes by tumor-associated myeloid cells can occur via ROS-mediated nitration (53, 69). Ag-independent mechanisms of myeloid cell-mediated inhibition of T cell function include local depletion of L-arginine via arginase upregulation or ROS production; release of immunomodulatory cytokines, such as TGF- β (51, 66); local cysteine depletion (70); and induction of L-selectin downregulation on T cells (71).

UPR involvement in Ag presentation

Jamora et al. (32) first showed that *Grp78*-deficient fibrosarcoma cells evoke a more robust T memory cell response, resulting in rejection of tumor cells with low immunogenicity.

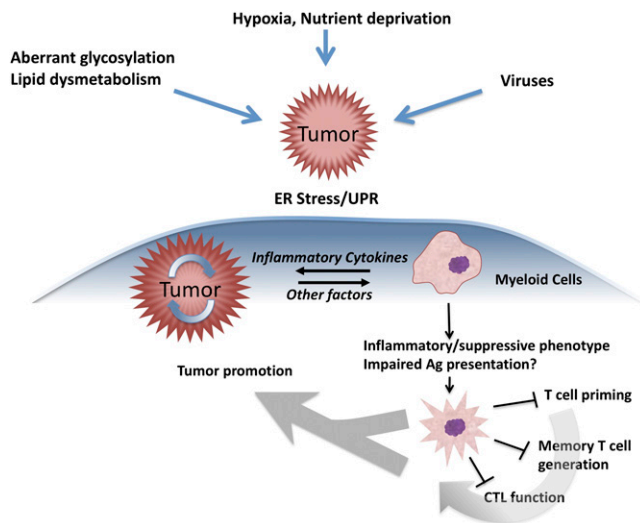


FIGURE 1. Relationships linking various ER stressors, the UPR, tumor cells, and immune cells in the microenvironment. The umbrella (blue shading) represents the multifaceted influence of tumor ER stress/UPR on myeloid cells and elements of the adaptive T cell response. In this new framework, the UPR acts as a cell-intrinsic tumor prosurvival factor (circular arrow). ER-stressed tumor cells also may secrete cytokines and other factors that harness myeloid cells in the tumor microenvironment, imprinting them with an inflammatory/suppressive phenotype (see text for description) that facilitates tumor growth via T cell-dependent and -independent mechanisms (merging arrows).

This finding suggested the intriguing, but little-explored, possibility that UPR signaling in tumor cells can impinge upon tumor Ag presentation and the host antitumor immune response. We showed that, although B cells mounting an UPR following accumulation of a KDEL-retained protein in the ER upregulate MHC class II and costimulatory molecules (72), they present decreased levels of high-affinity peptide complexed to MHC class II. Similarly, mouse thymoma cells under palmitate or glucose deprivation-induced ER stress decrease transgenic OVA Ag presentation on MHC class I (73). These findings begin to suggest that APC under ER stress undergo remodeling of the processing machinery, yielding decreased presentation of high-affinity immunodominant peptides and increased presentation of subdominant or low-affinity peptides. This was the conclusion of a study in human cancer cells treated with the histone deacetylase inhibitor trichostatin A, which also upregulates ER stress response genes while diminishing tapasin (74), a chaperone molecule involved in quality control of MHC I/peptide complexes in the ER (75). The relationship between ER

stress and tapasin is underscored by findings demonstrating that specific induction of the UPR using thapsigargin, a highly potent inducer of ER stress, causes downregulation of tapasin transcription in lymphoma cells (72). Thus, a UPR response in tumor cells and in APC may have the effect of rearranging the hierarchy of the immunopeptidome.

UPR-linked effects on immunoregulatory signals

The UPR is linked to the production of several inflammatory, tumorigenic cytokines: IL-6, IL-23, and TNF- α . A microarray analysis of mouse lymphoma cells under in vitro pharmacological ER stress revealed transcriptional upregulation of multiple inflammatory genes, including *Il-6*, *Il-23p19*, *Tnf- α* , *Tlr2*, and *Cebpb* (72). Furthermore, the levels of in vivo ER stress, as measured by *Grp78* expression, correlate with *Il-6*, *Il-23p19*, and *Tnf- α* transcription in murine prostate cancer cells growing in a heterotopic transplantation model (76).

CHOP is necessary for IL-23 production by dendritic cells (77) and IL-6 and TNF- α by macrophages (78). Redundant roles for IRE1 α and PERK signaling in IL-6 and TNF- α production in macrophages have been reported (78, 79). Chromatin immunoprecipitation analysis also reveals that XBP-1s binds to the promoters of *Il-6* and *Tnf- α* ; congruently, *Ire1 α* - or *Xbp-1*-deficient macrophages display impaired IL-6 and TNF- α production in response to pharmacological ER stress and infectious TLR agonism (79). The UPR also synergizes with TLR4 agonism to result in robust IL-23 secretion by macrophages (80). Interestingly, the UPR in cancer cells also upregulates lipocalin 2 (81), an innate immune inflammatory molecule whose first described function is the prevention of iron scavenging by bacterial siderophores (82). Notably, spontaneous breast cancer in mice showed a decreased rate of progression in *Lcn2*-deficient mice (83, 84).

UPR-linked proinflammatory mediators (85) in the tumor microenvironment facilitate tumor growth and regulate immune function (3). For example, inhibition of NF- κ B by ablation of IKK β in liver macrophages results in loss of TNF- α and IL-6 production, which, in turn, impairs tumor growth (86). Macrophage-specific deletion of IKK β leads to decreased production of PGE $_2$ and IL-6, resulting in reduced incidence of colitis-associated colorectal tumors (87). In a model of lung cancer, IL-6 and TNF- α produced by myeloid cells in response to tumor-derived versican (88) drive tumor growth and progression in a TLR2-dependent manner. IL-23 produced by TAM blocks CD8 T cell infiltration into tumors (89) and upregulates Treg differentiation in the tumor mi-

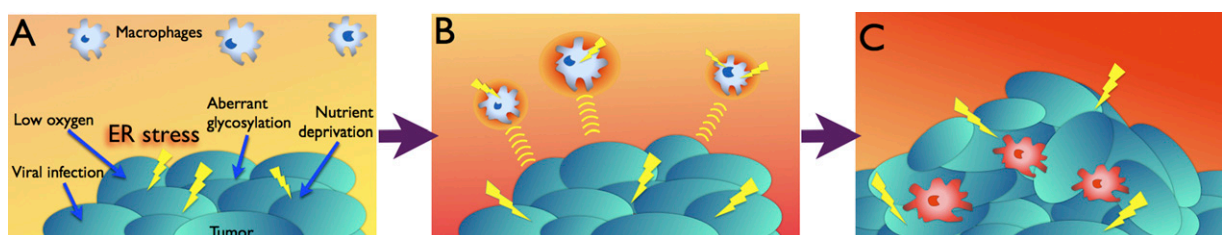


FIGURE 2. Dynamics of TERS from tumor cells to macrophages and consequences on tumor growth. *A*, Tumor cells experience ER stress (lightning bolts) as the result of a variety of causal noxae and, as a consequence, mount a UPR. *B*, In addition to initiating prosurvival signaling through the UPR, tumor cells release factors/signals to neighboring macrophages. In response, receiver macrophages undergo a UPR with attendant proinflammation (red aura). *C*, TERS-primed tumor-infiltrating macrophages (red) may facilitate tumor growth by amplifying tumorigenic inflammation and impairing T cell-mediated antitumor immunity, as suggested in Fig. 1.

croenvironment (90). The complex interrelationships between tumor cells and immune cells under the umbrella of ER stress are shown in Fig. 1.

Cell-extrinsic effects of the UPR on tumor-infiltrating immune cells

Thus far, the UPR has mostly been studied in the context of tumor-intrinsic signaling that sustains survival and proliferation. The new paradigm of a UPR-signaling umbrella under which proinflammation in the tumor microenvironment originates, together with the key role of inflammation in determining the phenotypic and functional complexity of TAM and TIDC, raises the intriguing possibility that the tumor UPR may impair host immune function in the tumor microenvironment in a cell-extrinsic manner. As a first step to investigate this hypothesis, we modeled the interaction between ER-stressed tumor cells and macrophages in the tumor microenvironment by culturing macrophages in the conditioned medium of murine cancer cells experiencing pharmacological or physiological ER stress *in vitro*. Surprisingly, macrophages treated in this manner upregulated UPR signaling, as well as the production of proinflammatory molecules, including IL-6, IL-23, TNF- α , CD86, MIP-1 α , and MIP-1 β . This “transmissible” ER stress (TERS) response is abrogated, in part, in TLR4-deficient macrophages, whereas signaling through either TLR2 or IL-6R, two likely candidates, is not involved. Additionally, macrophages exposed to tumor ER stress-conditioned medium upregulate arginase. The factor(s) responsible for TERS were also operational *in vivo* (91).

The factor(s) responsible for TERS have yet to be identified; studies of tumor cells deficient in each arm of the UPR will help to elucidate the molecular basis of the interplay between stressed tumor cells and macrophages. Recent findings showing UPR-mediated intercell trafficking of soluble UPR proteins (e.g., GRP78) (92) raise the intriguing possibility that elements of the UPR itself could communicate TERS. An alternative possibility implies a role for cyclooxygenase-2, which is upregulated during ER stress (93) and was shown to be responsible for the production of factors that subvert macrophages in the tumor microenvironment (61). In addition, future studies will have to address whether this new phenomenon is operational in spontaneously growing tumors. Furthermore, although the proposed immunosuppressive sequelae of TERS may be effected by already-known APC defects and/or immunoregulatory molecules (e.g., cytokines, chemokines, and small molecules), the novel role of the tumor UPR as the initiator of these events remains intriguing.

Thus, a new picture emerges in which microenvironment-induced ER stress in tumor cells initiates a tumorigenic, proinflammatory cascade that can be propagated to infiltrating macrophages, which concomitantly assume a phenotype enabling them to inhibit T cell responses. This phenotype fulfills many of the inflammatory/suppressive criteria observed in tumor-infiltrating myeloid cells. The cell-extrinsic influences of tumor ER stress on macrophages is shown in Fig. 2.

The consequences of tumor UPR-mediated derangement of APC, such as macrophages and dendritic cells, in the tumor microenvironment remain unknown. It is possible that, via its cell-extrinsic cues, the tumor UPR may ultimately manifest its consequences in defective T cell priming. As noted above, one consequence of ER stress in APC is impaired peptide presentation in the presence of adequate costimulatory molecule

expression. One may speculate that this could represent a novel mechanism of inadequate T cell priming in addition to the classical model of T cell anergy due to an insufficient costimulatory “second signal.” T cell dysfunction could also occur via Ag-independent mechanisms because the tumor UPR affects the cytokine and metabolic profile of the APC. A little explored possibility is that the UPR may cause immunosuppression via the induction of Treg differentiation. It has been well documented that Treg facilitate tumor growth by suppressing Ag-specific adaptive T cell responses (94). Although the relationship between tumor ER stress and Treg differentiation is unknown, we reported the involvement of the UPR in *Il-10* transcription by differentiating human Treg (95). Future work will need to elucidate these possibilities.

Conclusions

The UPR is an adaptive cell-signaling pathway that aids eukaryotic cells in coping with conditions in which the protein-folding capacity of the ER is saturated or diminished. The tumor microenvironment harbors multiple metabolic ER stressors that induce a UPR in tumor cells, which is critical for tumor cell survival and proliferation and has been correlated with progression. Based on this evidence, the tumor UPR has been most studied as a cell-intrinsic mechanism by which tumor cells survive. Based on recent findings, we posit that the tumor ER stress response can aid tumor growth in a cell-extrinsic manner by inhibiting antitumor immunity via T cell-independent and -dependent mechanisms.

Because UPR-inducing stimuli, such as hypoxia and glucose deprivation, are unique to tumor microenvironment, UPR-signaling molecules represent cancer-specific targets with broad-spectrum applicability, because such stresses are found in all solid-tumor microenvironments. Such strategies could aim to induce ER stress while simultaneously curbing tumor cell UPR signaling, thus leading to tumor regression, as has been demonstrated (96). The new proposal of TERS, which implicates host cells’ role as receivers/amplifiers of a pathogenic tumor-derived UPR, further suggests that treatment strategies targeting the UPR in the tumor microenvironment may be doubly beneficial.

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Disclosures

The authors have no financial conflicts of interest.

References

- Virchow, R. K. L. 1865. *Die Krankhaften Geschwülste*. A. Hirschwald, Berlin.
- Schreiber, R. D., L. J. Old, and M. J. Smyth. 2011. Cancer immunoeediting: integrating immunity’s roles in cancer suppression and promotion. *Science* 331: 1565–1570.
- Mumm, J. B., and M. Oft. 2008. Cytokine-based transformation of immune surveillance into tumor-promoting inflammation. *Oncogene* 27: 5913–5919.
- Badovinac, V. P., K. A. Messingham, A. Jabbari, J. S. Haring, and J. T. Harty. 2005. Accelerated CD8+ T-cell memory and prime-boost response after dendritic-cell vaccination. *Nat. Med.* 11: 748–756.
- Feldman, D. E., V. Chauhan, and A. C. Koong. 2005. The unfolded protein response: a novel component of the hypoxic stress response in tumors. *Mol. Cancer Res.* 3: 597–605.
- Harding, H. P., M. Calton, F. Urano, I. Novoa, and D. Ron. 2002. Transcriptional and translational control in the mammalian unfolded protein response. *Annu. Rev. Cell Dev. Biol.* 18: 575–599.
- Schröder, M., and R. J. Kaufman. 2005. ER stress and the unfolded protein response. *Mutat. Res.* 569: 29–63.

8. Bertolotti, A., Y. Zhang, L. M. Hendershot, H. P. Harding, and D. Ron. 2000. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2: 326–332.
9. Yoshida, H., T. Matsui, A. Yamamoto, T. Okada, and K. Mori. 2001. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107: 881–891.
10. Lee, A. H., N. N. Iwakoshi, and L. H. Glimcher. 2003. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell Biol.* 23: 7448–7459.
11. Yamamoto, K., T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada, and K. Mori. 2007. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev. Cell* 13: 365–376.
12. Harding, H. P., Y. Zhang, and D. Ron. 1999. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397: 271–274.
13. Zinszner, H., M. Kuroda, X. Wang, N. Batchvarova, R. T. Lightfoot, H. Remotti, J. L. Stevens, and D. Ron. 1998. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.* 12: 982–995.
14. Fulda, S., A. M. Gorman, O. Hori, and A. Samali. 2010. Cellular stress responses: cell survival and cell death. *Int. J. Cell Biol.* 2010: 214074.
15. Heazlewood, C. K., M. C. Cook, R. Eri, G. R. Price, S. B. Tauro, D. Taupin, D. J. Thornton, C. W. Png, T. L. Crockford, R. J. Cornell, et al. 2008. Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med.* 5: e54.
16. Little, J. L., F. B. Wheeler, D. R. Fels, C. Koumenis, and S. J. Kridel. 2007. Inhibition of fatty acid synthase induces endoplasmic reticulum stress in tumor cells. *Cancer Res.* 67: 1262–1269.
17. He, B. 2006. Viruses, endoplasmic reticulum stress, and interferon responses. *Cell Death Differ.* 13: 393–403.
18. Drogat, B., P. Auguste, D. T. Nguyen, M. Boucheccareilh, R. Pineau, J. Nalbantoglu, R. J. Kaufman, E. Chevet, A. Bikfalvi, and M. Moenner. 2007. IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. *Cancer Res.* 67: 6700–6707.
19. Romero-Ramirez, L., H. Cao, D. Nelson, E. Hammond, A. H. Lee, H. Yoshida, K. Mori, L. H. Glimcher, N. C. Denko, A. J. Giaccia, et al. 2004. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. *Cancer Res.* 64: 5943–5947.
20. Bi, M., C. Naczki, M. Koritzinsky, D. Fels, J. Blais, N. Hu, H. Harding, I. Novoa, M. Varia, J. Raleigh, et al. 2005. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J.* 24: 3470–3481.
21. Scriven, P., N. J. Brown, A. G. Pockley, and L. Wyld. 2007. The unfolded protein response and cancer: a brighter future unfolding? *J. Mol. Med.* 85: 331–341.
22. Henning, T., M. Kraus, M. Brischwein, A. M. Otto, and B. Wolf. 2004. Relevance of tumor microenvironment for progression, therapy and drug development. *Anticancer Drugs* 15: 7–14.
23. Shiu, R. P., J. Pouyssegur, and I. Pastan. 1977. Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts. *Proc. Natl. Acad. Sci. USA* 74: 3840–3844.
24. Spiotto, M. T., A. Banh, I. Papanliou, H. Cao, M. G. Galvez, G. C. Gurtner, N. C. Denko, Q. T. Le, and A. C. Koong. 2010. Imaging the unfolded protein response in primary tumors reveals microenvironments with metabolic variations that predict tumor growth. *Cancer Res.* 70: 78–88.
25. Fernandez, P. M., S. O. Tabbara, L. K. Jacobs, F. C. Manning, T. N. Tsangaris, A. M. Schwartz, K. A. Kennedy, and S. R. Patierno. 2000. Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. *Breast Cancer Res. Treat.* 59: 15–26.
26. Uramoto, H., K. Sugio, T. Oyama, S. Nakata, K. Ono, T. Yoshimastu, M. Morita, and K. Yasumoto. 2005. Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance. *Lung Cancer* 49: 55–62.
27. Shuda, M., N. Kondoh, N. Imazeki, K. Tanaka, T. Okada, K. Mori, A. Hada, M. Arai, T. Wakatsuki, O. Matsubara, et al. 2003. Activation of the ATF6, XBP1 and grp78 genes in human hepatocellular carcinoma: a possible involvement of the ER stress pathway in hepatocarcinogenesis. *J. Hepatol.* 38: 605–614.
28. Xing, X., M. Lai, Y. Wang, E. Xu, and Q. Huang. 2006. Overexpression of glucose-regulated protein 78 in colon cancer. *Clin. Chim. Acta* 364: 308–315.
29. Daneshmand, S., M. L. Quek, E. Lin, C. Lee, R. J. Cote, D. Hawes, J. Cai, S. Groshen, G. Lieskovsky, D. G. Skinner, et al. 2007. Glucose-regulated protein GRP78 is up-regulated in prostate cancer and correlates with recurrence and survival. *Hum. Pathol.* 38: 1547–1552.
30. Pyрко, P., A. H. Schönthal, F. M. Hoffman, T. C. Chen, and A. S. Lee. 2007. The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. *Cancer Res.* 67: 9809–9816.
31. Zhuang, L., R. A. Scolyer, C. S. Lee, S. W. McCarthy, W. A. Cooper, X. D. Zhang, J. F. Thompson, and P. Hersey. 2009. Expression of glucose-regulated stress protein GRP78 is related to progression of melanoma. *Histopathology* 54: 462–470.
32. Jamora, C., G. Dennert, and A. S. Lee. 1996. Inhibition of tumor progression by suppression of stress protein GRP78/BiP induction in fibrosarcoma B/C10ME. *Proc. Natl. Acad. Sci. USA* 93: 7690–7694.
33. Dong, D., M. Ni, J. Li, S. Xiong, W. Ye, J. J. Virrey, C. Mao, R. Ye, M. Wang, L. Pen, et al. 2008. Critical role of the stress chaperone GRP78/BIP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. *Cancer Res.* 68: 498–505.
34. Fu, Y., S. Wey, M. Wang, R. Ye, C. P. Liao, P. Roy-Burman, and A. S. Lee. 2008. Pten null prostate tumorigenesis and AKT activation are blocked by targeted knockout of ER chaperone GRP78/BiP in prostate epithelium. *Proc. Natl. Acad. Sci. USA* 105: 19444–19449.
35. Romero-Ramirez, L., H. Cao, M. P. Regalado, N. Kambham, D. Siemann, J. J. Kim, Q. T. Le, and A. C. Koong. 2009. X box-binding protein 1 regulates angiogenesis in human pancreatic adenocarcinomas. *Transl. Oncol.* 2: 31–38.
36. Blais, J. D., C. L. Addison, R. Edge, T. Falls, H. Zhao, K. Wary, C. Koumenis, H. P. Harding, D. Ron, M. Holcik, and J. C. Bell. 2006. Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. *Mol. Cell Biol.* 26: 9517–9532.
37. Bonizzi, G., and M. Karin. 2004. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* 25: 280–288.
38. Deng, J., P. D. Lu, Y. Zhang, D. Scheuner, R. J. Kaufman, N. Sonenberg, H. P. Harding, and D. Ron. 2004. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol. Cell Biol.* 24: 10161–10168.
39. Jiang, H. Y., S. A. Wek, B. C. McGrath, D. Scheuner, R. J. Kaufman, D. R. Cavener, and R. C. Wek. 2003. Phosphorylation of the alpha subunit of eukaryotic initiation factor 2 is required for activation of NF-kappaB in response to diverse cellular stresses. *Mol. Cell Biol.* 23: 5651–5663.
40. Hu, P., Z. Han, A. D. Couvillon, R. J. Kaufman, and J. H. Exton. 2006. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol. Cell Biol.* 26: 3071–3084.
41. Yamazaki, H., N. Hiramatsu, K. Hayakawa, Y. Tagawa, M. Okamura, R. Ogata, T. Huang, S. Nakajima, J. Yao, A. W. Paton, et al. 2009. Activation of the Akt-NF-kappaB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. *J. Immunol.* 183: 1480–1487.
42. Luo, D., Y. He, H. Zhang, L. Yu, H. Chen, Z. Xu, S. Tang, F. Urano, and W. Min. 2008. AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. *J. Biol. Chem.* 283: 11905–11912.
43. Urano, F., X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H. P. Harding, and D. Ron. 2000. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287: 664–666.
44. Xue, X., J. H. Piao, A. Nakajima, S. Sakon-Komazawa, Y. Kojima, K. Mori, H. Yagita, K. Okumura, H. Harding, and H. Nakano. 2005. Tumor necrosis factor alpha (TNFalpha) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFalpha. *J. Biol. Chem.* 280: 33917–33925.
45. Zhang, K., X. Shen, J. Wu, K. Sakaki, T. Saunders, D. T. Rutkowski, S. H. Back, and R. J. Kaufman. 2006. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124: 587–599.
46. Sulciner, D. J., K. Irani, Z. X. Yu, V. J. Ferrans, P. Goldschmidt-Clermont, and T. Finkel. 1996. rac1 regulates a cytokine-stimulated, redox-dependent pathway necessary for NF-kappaB activation. *Mol. Cell Biol.* 16: 7115–7121.
47. Cullinan, S. B., and J. A. Diehl. 2006. Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. *Int. J. Biochem. Cell Biol.* 38: 317–332.
48. Malhotra, J. D., and R. J. Kaufman. 2007. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signal.* 9: 2277–2293.
49. Malhotra, J. D., H. Miao, K. Zhang, A. Wolfson, S. Pennathur, S. W. Pipe, and R. J. Kaufman. 2008. Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc. Natl. Acad. Sci. USA* 105: 18525–18530.
50. Mantovani, A., S. Sozzani, M. Locati, P. Allavena, and A. Sica. 2002. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23: 549–555.
51. Kusmartsev, S., and D. I. Gabrilovich. 2006. Role of immature myeloid cells in mechanisms of immune evasion in cancer. *Cancer Immunol. Immunother.* 55: 237–245.
52. Sica, A., and V. Bronte. 2007. Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. Invest.* 117: 1155–1166.
53. Kusmartsev, S., Y. Nefedova, D. Yoder, and D. I. Gabrilovich. 2004. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J. Immunol.* 172: 989–999.
54. Huang, B., P. Y. Pan, Q. Li, A. I. Sato, D. E. Levy, J. Bromberg, C. M. Divino, and S. H. Chen. 2006. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res.* 66: 1123–1131.
55. Chaux, P., N. Favre, M. Martin, and F. Martin. 1997. Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression in rats. *Int. J. Cancer* 72: 619–624.
56. Ostrand-Rosenberg, S., and P. Sinha. 2009. Myeloid-derived suppressor cells: linking inflammation and cancer. *J. Immunol.* 182: 4499–4506.
57. Almand, B., J. I. Clark, E. Nikitina, J. van Beynen, N. R. English, S. C. Knight, D. P. Carbone, and D. I. Gabrilovich. 2001. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J. Immunol.* 166: 678–689.
58. Gabrilovich, D. I., H. L. Chen, K. R. Girgis, H. T. Cunningham, G. M. Meny, S. Nadaf, D. Kavanaugh, and D. P. Carbone. 1996. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat. Med.* 2: 1096–1103.
59. Bunt, S. K., L. Yang, P. Sinha, V. K. Clements, J. Leips, and S. Ostrand-Rosenberg. 2007. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res.* 67: 10019–10026.
60. Song, X., Y. Krelin, T. Dvorkin, O. Bjorkdahl, S. Segal, C. A. Dinarello, E. Voronov, and R. N. Apte. 2005. CD11b+/Gr-1+ immature myeloid cells me-

- diate suppression of T cells in mice bearing tumors of IL-1 β -secreting cells. *J. Immunol.* 175: 8200–8208.
61. Rodriguez, P. C., C. P. Hernandez, D. Quiceno, S. M. Dubinett, J. Zabaleta, J. B. Ochoa, J. Gilbert, and A. C. Ochoa. 2005. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J. Exp. Med.* 202: 931–939.
 62. Sinha, P., V. K. Clements, A. M. Fulton, and S. Ostrand-Rosenberg. 2007. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* 67: 4507–4513.
 63. Otsuji, M., Y. Kimura, T. Aoe, Y. Okamoto, and T. Saito. 1996. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc. Natl. Acad. Sci. USA* 93: 13119–13124.
 64. Ataera, H., E. Hyde, K. M. Price, P. Stoitznern, and F. Ronchese. 2011. Murine melanoma-infiltrating dendritic cells are defective in antigen presenting function regardless of the presence of CD4CD25 regulatory T cells. *PLoS ONE* 6: e17515.
 65. Liu, Q., C. Zhang, A. Sun, Y. Zheng, L. Wang, and X. Cao. 2009. Tumor-educated CD11bhighIalow regulatory dendritic cells suppress T cell response through arginase I. *J. Immunol.* 182: 6207–6216.
 66. Norian, L. A., P. C. Rodriguez, L. A. O'Mara, J. Zabaleta, A. C. Ochoa, M. Cella, and P. M. Allen. 2009. Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. *Cancer Res.* 69: 3086–3094.
 67. Stoitznern, P., L. K. Green, J. Y. Jung, K. M. Price, H. Atarea, B. Kivell, and F. Ronchese. 2008. Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. *Cancer Immunol. Immunother.* 57: 1665–1673.
 68. Van Ginderachter, J. A., K. Movahedi, G. Hassanzadeh Ghassabeh, S. Meerschaut, A. Beschin, G. Raes, and P. De Baetselier. 2006. Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. *Immunobiology* 211: 487–501.
 69. Nagaraj, S., K. Gupta, V. Pisarev, L. Kinarsky, S. Sherman, L. Kang, D. L. Herber, J. Schneck, and D. I. Gabrilovich. 2007. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat. Med.* 13: 828–835.
 70. Srivastava, M. K., P. Sinha, V. K. Clements, P. Rodriguez, and S. Ostrand-Rosenberg. 2010. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res.* 70: 68–77.
 71. Hanson, E. M., V. K. Clements, P. Sinha, D. Ilkovitch, and S. Ostrand-Rosenberg. 2009. Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J. Immunol.* 183: 937–944.
 72. Wheeler, M. C., M. Rizzi, R. Sasik, G. Almanza, G. Hardiman, and M. Zanetti. 2008. KDEL-retained antigen in B lymphocytes induces a proinflammatory response: a possible role for endoplasmic reticulum stress in adaptive T cell immunity. *J. Immunol.* 181: 256–264.
 73. Granados, D. P., P. L. Tanguay, M. P. Hardy, E. Caron, D. de Verteuil, S. Meloche, and C. Perreault. 2009. ER stress affects processing of MHC class I-associated peptides. *BMC Immunol.* 10: 10.
 74. Pellicciotta, I., X. Cortez-Gonzalez, R. Sasik, Y. Reiter, G. Hardiman, P. Langlade-Demoyen, and M. Zanetti. 2008. Presentation of telomerase reverse transcriptase, a self-tumor antigen, is down-regulated by histone deacetylase inhibition. *Cancer Res.* 68: 8085–8093.
 75. Howarth, M., A. Williams, A. B. Tolstrup, and T. Elliott. 2004. Tapasin enhances MHC class I peptide presentation according to peptide half-life. *Proc. Natl. Acad. Sci. USA* 101: 11737–11742.
 76. Mahadevan, N. R., A. Fernandez, J. J. Rodvold, G. Almanza, and M. Zanetti. 2010. Prostate cells undergoing ER stress *in vitro* and *in vivo* activate transcription of pro-inflammatory cytokines. *J. Inflamm. Res.* 3: 99–103.
 77. Goodall, J. C., C. Wu, Y. Zhang, L. McNeill, L. Ellis, V. Saudek, and J. S. Gaston. 2010. Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc. Natl. Acad. Sci. USA* 107: 17698–17703.
 78. Chen, L., S. Jarujaron, X. Wu, L. Sun, W. Zha, G. Liang, X. Wang, E. C. Gurley, E. J. Studer, P. B. Hylemon, et al. 2009. HIV protease inhibitor lopinavir-induced TNF-alpha and IL-6 expression is coupled to the unfolded protein response and ERK signaling pathways in macrophages. *Biochem. Pharmacol.* 78: 70–77.
 79. Martinon, F., X. Chen, A. H. Lee, and L. H. Glimcher. 2010. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* 11: 411–418.
 80. DeLay, M. L., M. J. Turner, E. I. Klenk, J. A. Smith, D. P. Sowders, and R. A. Colbert. 2009. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. *Arthritis Rheum.* 60: 2633–2643.
 81. Mahadevan, N. R., J. Rodvold, G. Almanza, A. Fernandez Perez, M. C. Wheeler, and M. Zanetti. 2011. ER stress activates Lipocalin 2 upregulation in prostate cancer cells in an NF- κ B-dependent manner. *BMC Cancer* 11: 229.
 82. Goetz, D. H., M. A. Holmes, N. Borregaard, M. E. Bluhm, K. N. Raymond, and R. K. Strong. 2002. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol. Cell* 10: 1033–1043.
 83. Leng, X., T. Ding, H. Lin, Y. Wang, L. Hu, J. Hu, B. Feig, W. Zhang, L. Pusztai, W. F. Symmans, et al. 2009. Inhibition of lipocalin 2 impairs breast tumorigenesis and metastasis. *Cancer Res.* 69: 8579–8584.
 84. Berger, T., C. C. Cheung, A. J. Elia, and T. W. Mak. 2010. Disruption of the Lcn2 gene in mice suppresses primary mammary tumor formation but does not decrease lung metastasis. *Proc. Natl. Acad. Sci. USA* 107: 2995–3000.
 85. Zhang, K., and R. J. Kaufman. 2008. From endoplasmic-reticulum stress to the inflammatory response. *Nature* 454: 455–462.
 86. Pikarsky, E., R. M. Porat, I. Stein, R. Abramovitch, S. Amit, S. Kasem, E. Gutkovich-Pyest, S. Urieli-Shoval, E. Galun, and Y. Ben-Neriah. 2004. NF- κ B functions as a tumour promoter in inflammation-associated cancer. *Nature* 431: 461–466.
 87. Greten, F. R., L. Eckmann, T. F. Greten, J. M. Park, Z. W. Li, L. J. Egan, M. F. Kagnoff, and M. Karin. 2004. IKK β links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118: 285–296.
 88. Kim, S., H. Takahashi, W. W. Lin, P. Descargues, S. Grivennikov, Y. Kim, J. L. Luo, and M. Karin. 2009. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature* 457: 102–106.
 89. Langowski, J. L., X. Zhang, L. Wu, J. D. Mattson, T. Chen, K. Smith, B. Basham, T. McClanahan, R. A. Kastelein, and M. Oft. 2006. IL-23 promotes tumour incidence and growth. *Nature* 442: 461–465.
 90. Kortylewski, M., H. Xin, M. Kujawski, H. Lee, Y. Liu, T. Harris, C. Drake, D. Pardoll, and H. Yu. 2009. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. *Cancer Cell* 15: 114–123.
 91. Mahadevan, N. R., J. Rodvold, H. Sepulveda, S. Rossi, A. F. Drew, and M. Zanetti. 2011. Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. *Proc. Natl. Acad. Sci. USA* 108: 6561–6566.
 92. Ni, M., Y. Zhang, and A. S. Lee. 2011. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. *Biochem. J.* 434: 181–188.
 93. Hung, J. H., I. J. Su, H. Y. Lei, H. C. Wang, W. C. Lin, W. T. Chang, W. Huang, W. C. Chang, Y. S. Chang, C. C. Chen, and M. D. Lai. 2004. Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF- κ B and pp38 mitogen-activated protein kinase. *J. Biol. Chem.* 279: 46384–46392.
 94. Sakaguchi, S., T. Yamaguchi, T. Nomura, and M. Ono. 2008. Regulatory T cells and immune tolerance. *Cell* 133: 775–787.
 95. Franco, A., G. Almanza, J. C. Burns, M. Wheeler, and M. Zanetti. 2010. Endoplasmic reticulum stress drives a regulatory phenotype in human T-cell clones. *Cell Immunol.* 266: 1–6.
 96. Lee, A. H., N. N. Iwakoshi, K. C. Anderson, and L. H. Glimcher. 2003. Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. *Proc. Natl. Acad. Sci. USA* 100: 9946–9951.

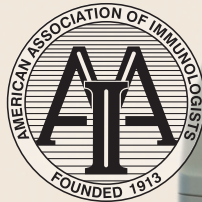
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