Chapter 18
Cell-Nonautonomous ER Stress-Mediated Dysregulation of Immunity by Cancer Cells

Jeffrey J. Rodvold, Navin R. Mahadevan, and Maurizio Zanetti

Abstract The immune surveillance hypothesis posits that neoantigens presented by tumor cells are detected by the immune system and eliminated, keeping tumor formation and growth at bay. Operationally this requires that tumor cells be taken up by local sentinels of the immune system, myeloid antigen presenting cells, which then proceed to present tumor associated antigens to T cells, resulting in specific rejection of tumor cells. Yet, one of the central unsolved paradoxes of tumor immunology is how the tumor escapes immune control which is reflected in the lack of effective autochthonous or vaccine-induced anti-tumor T cell responses.

In this chapter we discuss the emerging new idea that the endoplasmic reticulum (ER) stress response/unfolded protein response (UPR) activated in response to tumor microenvironmental *noxae*, acts not only as a key cell-intrinsic regulator of tumor growth and survival, but also as a central cell-extrinsic modulator of myeloid cell and T cell function. We will review the cellular and molecular basis of the anti-tumor immune response and the polarization of myeloid cells and T cells and place these into a UPR-centered perspective. We will also present the UPR as a cell-extrinsic regulator of anti-tumor immunity, effected by the newly-discovered "transmissible" ER stress.

Keywords ATF6: Activating transcription factor 6 • CHOP: CCAAT/-enhancer binding protein homologous protein • DC: Dendritic cell • ECM: Extracellular matrix • eIF2α: Eukaryotic translation initiation factor 2 alpha • EMT: Epithelial to mesenchymal transition • ER: Endoplasmic reticulum • FOXP3: Forkhead box 3 • GRP78: Glucose regulated protein 78 • HSR: Heat shock response • IDO: Indoleamine 2,3-dioxygenase • IL-: Interleukin • iNOS: Inducible nitric oxide synthase • IRE1α: Inositol requiring enzyme 1 alpha • LAG3: Lymphocyte activation gene 3 • MDSC: Myeloid derived suppressor cell • MEF: Mouse embryonic fibroblast • MHC: Major histocompatibility complex • NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells • OVA: Ovalbumin • PD-L1: Programmed cell death ligand 1 • PERK: Protein kinase-like endoplasmic reticulum kinase • PGE2: Prostaglandin E2 • TAM: Tumor associated macrophage • TCR: T-cell receptor • TERS: Transmissible endoplasmic

J.J. Rodvold • N.R. Mahadevan • M. Zanetti (✉)
Department of Medicine and Moores Cancer Center, The Laboratory of Immunology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0815, USA
e-mail: mzanetti@ucsd.edu

G.T. Wondrak (ed.), *Stress Response Pathways in Cancer*,
DOI 10.1007/978-94-017-9421-3_18

reticulum stress • TGFβ: Transforming growth factor beta • TIDC: Tumor infiltrating dendritic cell • TLR: Toll like receptor • UPR: Unfolded protein response

18.1 Introduction

Modern tumor immunology takes its roots in Burnet's immune surveillance hypothesis, which posits that the immune system is able to recognize tumor-associated antigens and act as a cell-extrinsic regulator of tumor growth (Burnet 1970). In humans, the immune surveillance hypothesis is supported by the detection of naturally-occurring T cell responses against self tumor antigens (Yotnda et al. 1998a, b; Molldrem et al. 2000; Nagorsen et al. 2003; Filaci et al. 2006), suggesting that central tolerance does not completely delete precursor T cells specific for a variety of self tumor antigens from the available repertoire. Studies in mice on sporadic cancer initiated through the rare spontaneous activation of a dormant oncogene showed that these tumors are in fact immunogenic and do not escape recognition by T cells but rather induce tolerance associated with the expansion of non-functional T cells (Willimsky and Blankenstein 2005). This is consistent with the observation that CD8 T cells generated by vaccination in melanoma patients are functionally heterogeneous and have a predominantly quiescent phenotype (Monsurro et al. 2002, 2004), reflecting perhaps a defective activation during priming. Thus, the complex landscape of anti-tumor T cell response depends on a delicate balance between activation of the residual T cell repertoire specific for self tumor antigens and mechanisms controlling the state of activation and function of T cells against these antigens.

Recently, emphasis has been placed on loss of immune surveillance subsequent to the disruption of the equilibrium at the tumor/immune interface mediated by tumor infiltrating myeloid cells (Balkwill and Mantovani 2001; Serafini et al. 2006). Virtually all solid tumors (carcinomas most notably) contain infiltrates of diverse leukocyte subsets including both myeloid- and lymphoid-lineage cells (Tlsty and Coussens 2006). Tumor-infiltrating leukocytes are rich in CD11b+ myeloid cells (Serafini et al. 2006), subsets of which produce factors that promote tumorigenesis by acting on tumor cells and immune cells. These secreted molecules include inflammatory cytokines that promote tumor cell growth and survival (IL-6, IL-23, and TNF-α, (Langowski et al. 2006; Kim et al. 2009) and for review see (Grivennikov et al. 2010; Mumm and Oft 2008), but also suppressive factors that inhibit T cell responses (e.g., IL-10, TGFβ, arginase – (*Arg1*), and indoleamine 2–3 dioxygenase (IDO)) (for review see (Gabrilovich et al. 2012)). Thus, tumor cells and the immune cells within the tumor microenvironment utilize "pro-inflammation" and "suppression" to exact a toll on adaptive T cell responses and facilitate tumor escape and growth (Van Ginderachter et al. 2006). However, the exact link between microenvironmental pro-inflammation/suppression and impairment of T cell function is not clearly understood.

Recent data from this laboratory provide a unifying view for this complex interplay, linking tumor cells, their microenvironment, leukocyte infiltration,

inflammation, and immune suppression. Our interpretative framework is based on the observation that the endoplasmic reticulum (ER) stress response in cancer cells causes the release of a factor(s) that itself recapitulates both ER stress in myeloid cells, macrophages and dendritic cells (DC), polarizing them to a pro-inflammatory/suppressive phenotype, ultimately impairing fundamental processes of the adaptive T cell response (Mahadevan et al. 2011a, 2012).

The endoplasmic reticulum (ER) is the initial checkpoint for the folding and modification of proteins that reside within the secretory pathway. The ER stress response, or unfolded protein response (UPR), is mediated by three initiator/sensor transmembrane molecules, inositol-requiring enzyme 1α (IRE1α), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6), which, in the unstressed state, are maintained in an inactive state through association with 78 kDa glucose-regulated protein (GRP78) (Walter and Ron 2011). Upon ER stress induction, GRP78 disassociates from the three UPR sensors, de-repressing them and allowing downstream signaling. Upon activation, PERK signals downstream effectors such as the growth arrest and DNA damage gene (GADD34) and the C/EBP homologous protein (CHOP), a regulator of inflammatory gene transcription and apoptotic cell machinery (Walter and Ron 2011). IRE1α is an endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding X-box-binding protein 1 (XBP-1). Spliced XBP-1 is a potent transcriptional activator that increases expression of a subset of the UPR-related genes involved in efficient protein folding, maturation, and degradation in the ER (Lee et al. 2003b).). In addition, under ER stress or forced autophosphorylation, IRE1α's RNase can cause endonucleolytic decay of many ER-localized mRNAs through a phenomenon termed regulated IRE1-dependent decay (RIDD) (Walter and Ron 2011). The transcription factor ATF6 activates UPR target genes that ameliorate the protein folding capacity of the ER (Walter and Ron 2011).

Cancer cells are consistently exposed to ER stress-inducing *noxae* within the tumor microenvironment. These include nutrient deprivation due in part to chaotic vasculature and highly active nutrient (i.e. glucose) consumption (aerobic glycolysis) (Warburg 1956), an imbalance between demand and supply of oxygen (hypoxia), an imbalance between the production of reactive oxygen and the cell's ability to readily detoxify the reactive intermediates (oxidative stress), and aberrant glycosylation (Heazlewood et al. 2008). Chronic viral infections (He 2006) which account for 18 % of cases of cancer world-wide (WHO International Agency for Cancer Research), als o cause ER stress.

It is the objective of this chapter to draw attention to the emerging paradigm that the cell-extrinsic consequences of tumor-borne UPR influence the dynamic equilibrium that exists at the tumor / immune interface. Specifically, we will discuss the anti-tumor immune response; its subversion via the co-existence of inflammation and suppression in the tumor microenvironment; the cell-intrinsic role of the UPR tumor adaptation and survival; cell-extrinsic mechanisms of tumor immune evasion; the UPR-mediated cell-extrinsic dysregulation of myeloid cells with impairment of antigen presentation and CD8 T cell activation; and therapeutic approaches targeting the tumor UPR.

18.2 The Anti-tumor Immune Response and Subversion

That immunity plays a protective role against spontaneous tumors dates back to 1912 and the pioneering work of G. Schone (cited in (Silverstein 1989)). In modern times this idea formed the basis of Burnet's immune surveillance hypothesis (Burnet 1970, 1971). New experimental data have provided for a revision of the original immune surveillance hypothesis (Schreiber et al. 2011) suggesting that tumor cell variants may not be completely eliminated by the immune system but instead enter into an equilibrium phase during which T cells constrain clinically undetectable occult tumor cells and edit tumor cell antigenicity and immunogenicity (Koebel et al. 2007). The resulting edited tumor cells possess reduced immunogenicity and begin to grow in an immunologically unrestrained manner.

18.2.1 The Anti-tumor Adaptive Immune Response

Adaptive anti-tumor T cell responses are based on the recognition of antigens expressed on the surface of tumor cells in association with molecules of the major histocompatibility complex (MHC). However, self tumor antigens use a variety of strategies to evade immune surveillance: tolerance/anergy, ignorance and active immunosuppression through soluble mediators. In addition, escape also occurs through immune suppression mediated by CD4 and CD8 regulatory T cells (Tregs) (Sakaguchi 2003; Sakaguchi et al. 2008), a class of cells increased in patients with malignancies and in tumor tissues (Zou 2006; Mougiakakos et al. 2010; Jacobs et al. 2012; Whiteside 2012). Studies in mice show that antigen specific tumor-infiltrating CD8 T lymphocytes display an activated phenotype but little cytotoxicity when transferred into tumor-bearing mice (Savage et al. 2008). Sporadic tumors in mice are immunogenic but induce tolerance associated with the expansion of non-functional T cells (Willimsky and Blankenstein 2005). T cells tolerant to self antigen return to a tolerant phenotype even after having resumed proliferation and function (Schietinger et al. 2012). This shows that tumor-initiated *active* regulation of the adaptive T cell response plays an important role in the lack of effectiveness of anti-tumor immunity.

18.2.2 Tumorigenic Cytokines in the Tumor Microenvironment

Inflammatory cytokines, often under the control of NF-κB, promote tumor cell survival, proliferation, and immune subversion. While oncogene activation in tumor cells can lead to cytokine production and secretion, the predominant source of tumorigenic inflammatory mediators are tumor-infiltrating myeloid cells (Grivennikov et al. 2010). For example, inhibition of NF–κB by ablation of IKKβ

in liver macrophages results in loss of TNF-α and IL-6 production, which in turn, impairs tumor growth (Pikarsky et al. 2004). Macrophage-specific deletion of IKKβ leads to decreased production of PGE_2 and IL-6, resulting in reduced incidence of colitis-associated colorectal tumors (Greten et al. 2004). Myeloid cells (macrophages and dendritic cells) of the lamina propria were found to be a key source of IL-6, which plays a crucial role in driving tumorigenesis in a mouse model of colitis-associated cancer (Grivennikov et al. 2009). In a model of lung cancer, IL-6 and TNF-α produced by myeloid cells in response to tumor-derived versican (Kim et al. 2009) drive tumor growth and progression in a TLR2-dependent manner. IL-6 production by hepatocellular carcinoma (HCC) progenitor cells and Kupffer cells in early dysplastic lesions in a model of carcinogen-driven liver carcinogenesis promotes progression to HCC (Naugler and Karin 2008; Akers et al. 2013).

IL-23 produced by tumor associated macrophages (TAMs) blocks CD8 T cell infiltration into skin tumors (Langowski et al. 2006) and upregulates T regulatory cell differentiation in the melanoma microenvironment (Kortylewski et al. 2009). In a mouse model of spontaneous colon cancer, bacterial TLR ligands penetrate the colonic mucosal barrier and promote IL-23 production by adenoma-infiltrating myeloid cells, ultimately leading to tumor outgrowth likely via induction of downstream tumor-promoting cytokines, including IL-17 and IL-6 (Grivennikov et al. 2010). In addition, tumor-associated myeloid dendritic cells are a key source of IL-23 in a mouse model of lung cancer, and a neutralizing IL-23 antibody combined with agonistic CD40 antibodies reduces primary fibrosarcoma and metastatic melanoma tumor burden (von Scheidt et al. 2014).

The TGFβ family of cytokines, has different roles at different stages of tumorigenesis within in the tumor microenvironment. The source of TGFβ can be tumor cells themselves, especially early in tumor growth; however infiltrating myeloid cells are a major TGFβ source later during tumor progression (reviewed in (Massague 2008)). Early during tumor growth, TGFβ restrains tumorigenesis via cell-intrinsic and cell-extrinsic mechanisms: (1) repression of the cell cycle and induction of cell cycle inhibitors, (2) promotion of cellular differentiation and senescence, (3) activation apoptotic machinery, (4) suppression of autocrine and paracrine mitogenic signaling in neighboring stromal fibroblasts, and (5) inhibition of innate and adaptive immune cell function and tumorigenic cytokine production (reviewed in (Massague 2008; Pickup et al. 2013)).

During tumor progression, however, malignant cells inactivate TGFβ signaling and can co-opt other tumorigenic functions of TGFβ signaling, including extracellular matrix (ECM) degradation via matrix metalloproteinase production, epithelial-to-mesenchymal transition (EMT) (Chaffer and Weinberg 2011), and stimulation of angiogenesis. In this context, TGFβ can promote tumorigenic inflammatory and immunosuppressive effects in invading immune cells. For instance, TGFβ and IL-6 drive CD8 and CD4 T cell differentiation to the Tc17 and Th17 phenotypes, which promote tumor growth in the correct context via promotion of angiogenesis and tumor cell proliferation (reviewed in (Pickup et al. 2013). Inversely, TGFβ signaling directs polarization of tumor-associated myeloid cells to a suppressive phenotype, which inhibits T cell function *in vitro* and perhaps *in vivo* (reviewed in (Pickup et al.

2013; Mao et al. 2014)). In addition, TGFβ signaling in CD8+ T cells represses expression of the Natural killer group 2, member D (NKG2D) receptor and inhibits cytolytic activity (Friese et al. 2004; Thomas and Massague 2005).

It should be noted that, while the tumorigenic role of various inflammatory mediators, including NF-kB, IL-6, IL-23, and TGFβ, have been well illustrated, the tumor-mediated mechanism of their production in the tumor microenvironment, notably, by tumor-associated myeloid cells, remains less clear.

18.2.3 Tumor-Associated Myeloid Cells

Virtually all solid tumors (carcinomas most notably) contain infiltrates of diverse leukocyte subsets, mainly myeloid cells (Tlsty and Coussens 2006), which express the CD11b+surface marker (Serafini et al. 2006; Ruffell et al. 2012) and have been stratified into tumor-associated macrophages (TAM) ($F4/80^{+}/Gr1^{+}$), myeloid-derived suppressor cells (MDSC) ($Gr\text{-}1^{+}$) and tumor infiltrating myeloid dendritic cells ($CD11c^{+}$). As a whole, myeloid cells that infiltrate solid tumors are key players in the cell-extrinsic regulation of tumor growth, often producing a variety of pro-tumorigenic factors that effectively modify the tumor/immune cell landscape. Because of their ability to inhibit T cell responses *in vitro* and *in vivo* (Kusmartsev et al. 2004; Huang et al. 2006), and the initial characterization of their phenotype as $IL\text{-}10^{+}/IL\text{-}12^{-}$ coupled with low levels of costimulatory molecules and antigen presentation machinery, it was proposed that tumor-associated CD11b+/Gr1+ myeloid cells possessed an anti-inflammatory and suppressive (M2) phenotype (Mantovani et al. 2002). Tumor infiltrating dendritic cells (TIDC) were first characterized as having an immature phenotype characterized by low levels of MHC Class I and II, and co-stimulatory molecule (CD86/CD80) expression, which was assumed to be responsible for the dysfunctional T cell priming and induction of anergy observed by immature DC in non-tumor systems or in the peripheral blood of cancer patients (Chaux et al. 1997; Gabrilovich et al. 1997; Probst et al. 2003; Friese et al. 2004).

More recently, however, evidence has accumulated that suggests that the tumorigenic phenotype of myeloid cells is concomitantly pro-inflammatory and actively suppressive (for a extensive review on the topic, see (Ostrand-Rosenberg and Sinha 2009)). For instance, in tumor-associated myeloid cells, generation of reactive oxygen species crucial for the inhibition of T cell responses can occur via arginase, a classical M2 marker, but also via iNOS, an inflammatory (M1) marker (Otsuji et al. 1996; Kusmartsev et al. 2004). Furthermore, tumor-derived myeloid cells produce inflammatory cytokines that play key roles in tumor growth and in regulating anti-tumor immunity (Mumm and Oft 2008; Grivennikov et al. 2010). More recently, it has been found that TIDC in melanoma, lung carcinoma, ovarian cancer, and breast cancer express high levels of MHC Class I/II, CD80, and CD86, yet they still inhibit anti-tumor CD8 T cell responses *in vitro* and *in vivo* due to a combination of inadequate antigen presentation, arginase production, or PD-L1 expression (Stoitzner et al. 2008; Liu et al. 2009; Norian et al. 2009; Engelhardt

et al. 2012; Scarlett et al. 2012). For example, in a murine model of ovarian carcinoma, as well as in human ovarian tumor samples, TIDC with a "regulatory" phenotype hallmarked by expression of MHC II, CD86, and DEC205, promote tumor outgrowth by suppressing T cell function within the tumor via IL-6 activity, PD-L1, Arginase I, respectively (Scarlett et al. 2012; Tesone et al. 2013).

Large cohort studies in breast cancer patients have shown that the presence of macrophages expressing CD68 correlates with poor prognostic features (Denardo et al. 2011), increased angiogenesis (Cavanagh et al. 2005) and decreased disease-free survival (Cairns et al. 2011). In addition, presence of increased numbers of CD68+ macrophages in tumor stroma in patients with non-small-cell lung carcinoma (NSCLC) correlated with poorer overall survival (Welsh et al. 2005; Kawai et al. 2008; Dai et al. 2010).

18.3 Co-existence of Inflammation and Suppression in the Tumor Microenvironment and the Cell-Intrinsic Contribution of the UPR to Tumor Progression

There is increasing evidence that the tumor/immune interplay is important in tumor growth and invasiveness (Hanahan and Weinberg 2011), and that local inflammation (Balkwill and Mantovani 2001; Balkwill et al. 2005; Grivennikov et al. 2010; Cou ssens et al. 2013) plays a key role. The vast majority (95 %) of cancers display, and likely result from, somatic, as opposed to germline mutations (Vogelstein et al. 2013). Since inflammation has been linked to genomic instability (Tili et al. 2011), inflammation-promoting conditions (obesity, diet, stress, viruses) could serve as precondition to cancer growth and progression. In addition, infiltrating myeloid cells and T cells have the capacity to produce a variety of pro-tumorigenic factors that effectively modify the tumor/immune cell landscape. For example, tumorigenic effects have been associated with pro-inflammatory cytokines (IL-6, IL-23, TNF-α and MIF), but, also with anti-inflammatory cytokines (IL-10, TGFβ) and molecules with immune suppressive function (arginase, peroxynitrite and indoleamine 2–3 dioxygenase) (for review see (Gabrilovich et al. 2012)). Furthermore, through the secretion of a variety of cytokines, tumor-infiltrating myeloid cells also contribute to tumor angiogenesis (Shojaei et al. 2007; Kujawski et al. 2008; Chen and Bonaldo 2013).

How myeloid cells become causative for tumor growth and progression and what tumor-derived cues determine their polarization is still poorly understood. Even more perplexing is the apparent paradox that the tumor microenvironment is at once pro-inflammatory and anti-inflammatory, suggesting the possibility that myeloid cells may, at a certain point, possess a "mixed" pro-inflammatory/suppressive phenotype (Van Ginderachter et al. 2006). Hereunder we will present evidence for a unifying mechanistic interpretation of this paradox.

18.3.1 Cell-Intrinsic Role of UPR in Tumor Adaptation and Progression

The starting point of our new hypothesis is evidence implicating ER stress and the UPR in tumorigenesis, cancer growth, and progression. Primary human tumor cells of several origins, including breast (Fernandez et al. 2000), lung (Uramoto et al. 2005), liver (Shuda et al. 2003), colon (Xing et al. 2006), prostate (Daneshmand et al. 2007), and brain (Pyrko et al. 2007) have been shown to upregulate UPR pathways, whereas peritumoral areas do not. Additionally, in primary human melanoma, liver, and breast cancer specimens, the level of GRP78 positively correlates with tumor progression (Fernandez et al. 2000; Shuda et al. 2003; Zhuang et al. 2009). GRP78 has also been detected on the surface of tumor cells of diverse histological origin (Arap et al. 2004; Davidson et al. 2005; Misra et al. 2006).

The conditional homozygous knockout of *Grp78* in the prostate of mice with *Pten* inactivation protects against cancer growth (Fu et al. 2008) and inactivation of a *Grp78* allele in the *MMTV-PyT* murine model of breast cancer yields significantly decreased breast tumor proliferation, survival, and angiogenesis compared to *Grp78*+/+, *PyT* mice (Dong et al. 2008). Lastly, proliferating and dormant cancer cells in which *Grp78* is upregulated are resistant to chemotherapy (Reddy et al. 2003; Ranganathan et al. 2006; Chang et al. 2007; Fu et al. 2007; Pyrko et al. 2007).

Transformed mouse fibroblasts deficient in *Xbp1* 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, mouse embryonic fibroblasts (MEFs) expressing a siRNA against *Xbp-1* lead to tumors that are smaller and exhibit decreased angiogenesis as compared to tumors generated by control cells when injected into mice (Romero-Ramirez et al. 2004, 2009). Similarly, siRNA 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 IRE1α mutant rescues xenograft angiogenesis (Romero-Ramirez et al. 2004, 2009). Additionally, human glioma cells expressing a dominant-negative IRE1α mutant display a decreased growth rate and impaired angiogenesis when orthotopically transplanted into immunodeficient mice (Drogat et al. 2007).

The inactivation of PERK or a dominant-negative PERK in tumor cells, results in tumors that are smaller and less aggressive than their normal counterparts when implanted into mice (Bi et al. 2005). And finally, tumor cells cultured under hypoxic/anoxic conditions and transformed cells in hypoxic areas of tumors activate ER stress. Inactivation of PERK results in impaired tumor cell survival under hypoxic conditions *in vitro*, and decreased tumor growth and angiogenesis *in vivo* (Bi et al. 2005; Blais et al. 2006). Taken together, these results underscore the key contribution of UPR in the adaptation and progression of solid tumors of diverse origins.

18.3.2 UPR Involvement in Regulation of Inflammatory Mediators

Besides promoting cellular adaptation to increased un/misfolded protein burden, the UPR activates a pro-inflammatory cascade with tumor-promoting and cell-survival effects. One of the key inflammatory regulators inducible by the UPR is the transcription factor NF-κB (Bonizzi and Karin 2004). Each of the three UPR signaling pathways activates NF-κB translocation to the nucleus via distinct mechanisms. PERK-mediated translational inhibition reduces the ratio of the IκB to NF-κB thus permitting the nuclear migration of NF-κB and transcription of downstream inflammatory genes (Jiang et al. 2003; Deng et al. 2004). Upon auto-phosphorylation, IRE1α forms a complex with tumor-necrosis factor-α (TNF-α)-receptor-associated factor 2 (TRAF2) at its cytosolic domain, and the IRE1α-TRAF2 complex mediates direct IκB phosphorylation via IκB kinase (IKK), which leads to NF-κB activation (Hu et al. 2006). Lastly, ATF6 was shown to participate in NF-κB activation in an AKT-dependent manner (Yamazaki et al. 2009).

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 reveals transcriptional upregulation of multiple inflammatory genes, including *Il-6*, *Il-23p19*, *Tnf-α*, *Tlr2*, and *Cebpb* (Wheeler et al. 2008). 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 (Mahadevan et al. 2010).

CHOP is necessary for IL-23 production by dendritic cells (Goodall et al. 2010), and IL-6 and TNF-α by macrophages (Chen et al. 2009). Redundant roles for IRE1α and PERK signaling in IL-6 and TNF-α production in macrophages have been reported (Chen et al. 2009; Martinon et al. 2010). ChIP analysis also reveals that XBP-1s binds to the promoters of the *Il-6* and *Tnf-α*; congruently, Ire1α- or *Xbp1-deficient* macrophages display impaired IL-6 and TNF-α production in response to pharmacological ER stress and infectious TLR agonism (Martinon et al. 2010). The UPR also synergizes with TLR4 agonism to result in robust IL-23 secretion by macrophages (DeLay et al. 2009). Interestingly, murine tumor-associated macrophages deficient in the UPR effector chaperone Grp94, have reduced inflammatory cytokine (IL-1β, IL-6, IL-17A, IL-17F, IL-18, IL-22, IL-23, IFN-γ, and TNF-α) production, which correlates with decreased colitis-associated tumor burden (Morales et al. 2014). Thus, the UPR is a key regulator of the production of inflammatory mediators.

18.3.3 UPR-Mediated Dysregulation of Antigen Presentation

In addition, evidence suggests that UPR signaling in tumor cells and in antigen-presenting cells can impinge upon antigen presentation. While B cells mount a UPR following accumulation of a KDEL-retained protein in the ER upregulate

MHC Class II and costimulatory molecules, they present decreased levels of high affinity peptide complexed to MHC Class II (Wheeler et al. 2008). 293T cells overexpressing an ER stress-inducing misfolded protein or constitutively active ATF6 or XBP-1s display decreased levels of MHC Class I (de Almeida et al. 2007). Similarly, mouse thymoma cells that undergo ER stress through palmitate treatment or glucose deprivation decrease antigen presentation on MHC Class I (Granados et al. 2009). Induction of UPR genes in lymphoma cells with thapsigargin or the histone deacetylase inhibitor, trichostatin A, is associated with the transcriptional downregulation of tapasin (Pellicciotta et al. 2008; Wheeler et al. 2008), a chaperone molecule involved in quality control of MHC I/peptide complexes in the ER (Howarth et al. 2004). Moreover, IRE1α-mediated signaling upregulates the expression of miR346, which in turn downregulates the protein transporter associated with antigen processing 1 (TAP1), ostensibly decreasing MHC Class I-associated antigen presentation (Bartoszewski et al. 2011). Most recently, it was shown that in CD8α$^+$ dendritic cells, IRE1α regulated dependent decay (RIDD) of mRNAs coding for components of the MHC Class I presentation pathway, including tapasin, leads to dysfunctional cross presentation and cross-priming of antigen-specific CD8+ T cells (Osorio et al. 2014). These findings suggest that cancer cells and antigen presenting cells mounting a UPR undergo remodeling of the processing machinery yielding decreased presentation of high affinity immunodominant peptides.

18.4 Cell-Extrinsic Polarization of Myeloid Cells via the Tumor UPR

Recent reports have begun suggest that the UPR can regulate anti-tumor immunity via modulation of myeloid cell function. For instance, hyperploid cancer cells translocate the ER chaperone, calreticulin, to the cell membrane in a UPR-dependent manner, promoting tumor cell phagocytosis by myeloid antigen presenting cells, ultimately possibly initiating a cellular immune response against hyperploid cancer cells (Senovilla et al. 2012).

On the other hand, we uncovered a previously unappreciated cell-extrinsic effect of the tumor UPR: its transmission to myeloid cells, i.e., macrophages and DC (Mahadevan et al. 2011b, 2012), which culminates in subversion of anti-tumor immunity. This new phenomenon, "transmissible" ER stress (TERS), was discovered while investigating the effects of conditioned medium from ER stressed murine tumor cells (e.g., prostate, melanoma, and lung carcinoma) on bone marrow-derived macrophages and dendritic cells (DC). In these experiments, cancer cells were stressed using thapsigargin, a sesquiterpene lactone canonical ER stress inducer that inhibits the sarco/endoplasmic reticulum Ca^{2+} ATPase, or glucose starvation. We found that bone marrow-derived macrophages and DC both function as receivers of TERS. The changes imparted on receiver cells include (i) the induction of a global ER stress response (e.g., the transcriptional upregulation of *Grp78*, *Xbp-1s*, and

Chop); (ii) the upregulation of pro-inflammatory/pro-tumorigenic cytokines (i.e., *Il-6*, *Il-23p19*, and *Tnf-α*); (iii) the increased secretion of other pro-tumorigenic cytokines/chemokines (TGFβ, MIP-1α, MIP-1β); and (iv) the upregulation of Arginase 1 (Mahadevan et al. 2011b, 2012), an enzyme that suppresses T cell function (Bronte and Zanovello 2005). TERS had no effect on IL-10 in myeloid cells. In addition, TERS-imprinted myeloid cells do not upregulate GR-1, distinguishing their phenotype from that of classical MDSC (Gabrilovich et al. 2012). We found that PD-L1, the ligand for the T cell immune-inhibitory PD-1 receptor (Freeman et al. 2000), is somewhat increased by TERS in myeloid DC (Mahadevan et al. 2012). TERS-imprinted myeloid DC rapidly change morphology, acquiring morphological characteristics of activated DC, including increased size and elongated dendrites. They also upregulate expression of MHC Class I and Class II, and the costimulatory molecules CD86, CD80 (classical markers of immune activation), and, to a lesser extent, CD40 (Mahadevan et al. 2012). (Cullen et al. 2013) confirmed that TERS from breast cancer cells can remodel macrophage phenotype similar to the pro-inflammatory/suppressive one described above, and, in addition, demonstrated that TERS-imprinted macrophages secrete VEGF. The general phenotypic features of $CD11b^{+}$ cells, macrophages and DC, upon TERS imprinting are summarized in Fig. 18.1. In addition, transmission of ER stress was reduced in TLR4KO macrophage receiver cells, suggesting that TLR4 senses, at least in part, the transmission of tumor borne stress.

Taking into account the current evidence, the cell-extrinsic effects of the tumor UPR appear to be contrasting: on the one hand promoting immune surveillance of

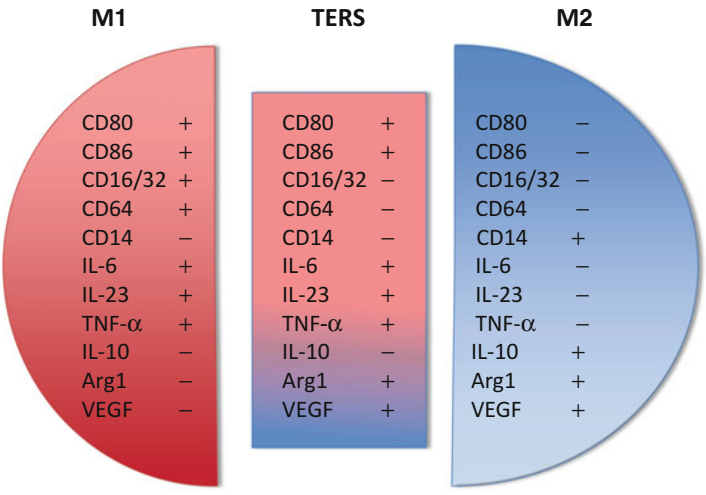

Fig. 18.1 The pro-inflammatory/suppressive phenotype of TERS-imprinted myeloid cells. The characteristics of TERS-imprinted bone marrow-derived macrophages (*middle*) are compared with those of canonical characteristics of M1 (*left*) and M2 (*right*) macrophages (Adapted from Zanetti (2013))

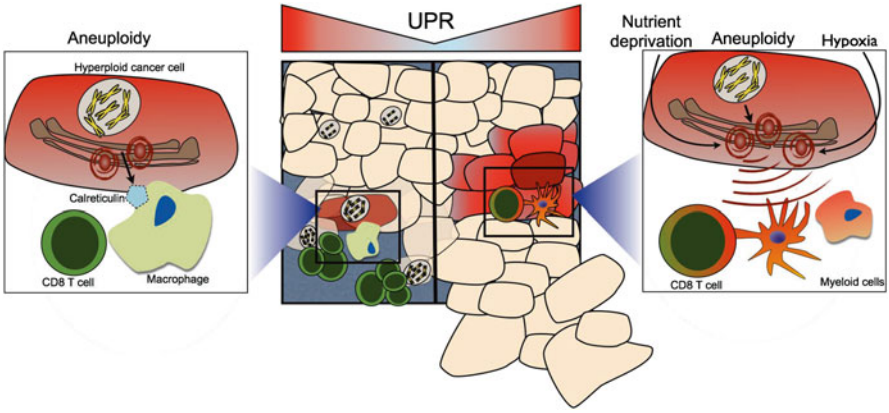

Fig. 18.2 Janus-faced effects of the tumor UPR on anti-tumor T cell immunity. (*Left and left inset*) Hyperploid cancer cells induce a UPR-dependent translocation of calreticulin to the cell surface where it serves as a "eat-me" signal for phagocytic cells (macrophages and dendritic cells). These take up tumor cell debris and apoptotic bodies, and present tumor antigens to T cells, inducing an anti-tumor immune response, which leads to the selective elimination of hyperploid cancer cells (Senovilla et al. 2012). (*Right panel and right inset*) The tumor UPR polarizes infiltrating myeloid cells to a pro-inflammatory/suppressive phenotype characterized by inefficient antigen presentation and CD8 T cell cross-priming, ultimately derailing anti-tumor T cell immunity, leading to tumor growth facilitation (Mahadevan et al. 2012) (From Mahadevan et al. (2013) with permission)

hyperploid tumor cells via ER stress-enforced calreticulin expression (Senovilla et al. 2012), while, on the other, imprinting macrophages and dendritic cells with a pro-inflammatory/suppressive, mature phenotype with functional abnormalities with respect to antigen processing and presentation to T cells, as will be discussed below. To reconcile these seemingly contrasting effects, we suggested (Mahadevan et al. 2013) that the tumor UPR response may fulfill both functions, perhaps promoting cellular immunity against hyperploid cells on the one hand early during tumorigenesis, while ultimately undermining the immune response against cancer cells. That clinical tumors samples exhibit heterogeneous ploidy (Ohyama et al. 1990), and tetraploidy is key event in the progression of diverse histological subtypes (Davoli and de Lange 2011), suggest that this might indeed be the case. The seemingly Janus-faced cell-extrinsic role of the tumor UPR is shown in Fig. 18.2 (adapted from (Mahadevan et al. 2013)).

18.5 TERS Inhibits Antigen Presentation and CD8 T Cell Priming by Bone Marrow-Derived DC

In a series of experiments, we demonstrated that TERS impacts adversely upon myeloid DC cross-presentation and cross-priming (Mahadevan et al. 2012), two events associated with the induction of CD8 T cell-mediated immunity.

18.5.1 Effects on Cross-Presentation

To study cross-presentation we used a system in which the ovalbumin (OVA) SIINFEKL peptide bound to the H2-K^b molecule can be detected by flow cytometry using a monoclonal antibody. Reproducibly, OVA-fed, TERS-imprinted DC have reduced display of the SIINFEKL/H2-K^b complex at the cell surface, while the expression of MHC Class I molecules remains constant or even increased over that of OVA-fed control DC. Thus, TERS down-regulates cross-presentation.

18.5.2 Effects on Cross-Priming/T Cell Activation

To study cross-priming we used CD8 T cells from OT-I mice whose T cell receptor (TCR) is specific for the SIINFEKL/H2-K^b complex. In this model, OVA-fed bone marrow-derived DC efficiently induce both the activation and proliferation of OT-I CD8 T cells. When OT-I CD8 T cells are co-cultured with OVA-fed TERS-imprinted bone marrow-derived DC, however, while being activated, they proliferate poorly. On average, the majority (>70 %) of CD8 T cells are activated/non-dividing T cells. PD-1, a marker of "exhausted" T cells, is not upregulated. Importantly, antigen-specific CD8 T cells activated by TERS-imprinted myeloid DC show decreased ability to kill target cells [our unpublished data].

Importantly, we found that TERS-imprinted bone marrow-derived DC could exert dominant suppression over cross-priming by normal bystander antigen presenting cells. When TERS-imprinted bone marrow-derived DC, with or without antigen, are added to co-cultures naïve CD8 T cells and antigen-fed control bone marrow-derived DC, the proliferation of CD8 T cells is suppressed.

18.5.3 Mechanisms of Cross-Priming Defect

Efforts to restore the proliferative defect showed the following. (i) The addition of excess SIINFEKL antigen (1 μg/mL), rescued proliferation in OT-I T cells cross-primed by TERS-imprinted bone marrow-derived DC. (ii) The addition of exogenous IL-2 during cross-priming failed to rescue OT-I T cell proliferation, ruling out the possibility of classical anergy (Beverly et al. 1992). (iii) Removal from the co-culture containing TERS-imprinted bone marrow-derived DC partially restored T cell proliferation, although with fewer cell divisions, suggesting the importance of cell-cell contact. (iv) Whereas the addition of exogenous L-arginine to the co-culture did not improve T cell proliferation, the addition of L-norvaline, a competitive inhibitor of arginase, rescued it in great part (80 %). Taken together, these results suggest that tumor UPR-mediated myeloid cell-derived arginase activity and impaired cross-presentation together contribute to the T cell proliferative defect

observed. Interestingly, however, addition of L-norvaline did not rescue T cell proliferation caused by dominant suppression.

18.5.4 TERS-Imprinted Myeloid DC in Context

Modeling the cell-extrinsic influence of the tumor UPR showed that TERS-imprinted BMDC are phenotypically mature, upregulate costimulatory molecules, have diminished cross-presentation capacity, and exert suppressive activity over CD8 T cells and bystander DC. Tolerogenic DC have been described in various systems (Steinman et al. 2003), and were initially defined in the periphery as steady-state, immature cells able to present antigen that suppress T cell activity because of inadequate co-stimulatory capacity (Gabrilovich et al. 1997; Steinman and Nussenzweig 2002). In the microenvironment of solid tumors of several histological types, infiltrating dendritic cells can be identified that display an immature phenotype with decreased MHC Class II, CD80, CD86, and CD83 expression, with presumed passive T cell inhibitory activity (Chaux et al. 1997; Bell et al. 1999; Pinzon-Charry et al. 2005; Tesone et al. 2013).

However, evidence has begun to accumulate ascribing active immunosuppressive activity via several mechanisms (e.g. arginase, IDO, and PD-L1 activity; for review, see (Tesone et al. 2013)) to phenotypically mature, so-called "regulatory" dendritic cells (Tesone et al. 2013). TERS-imprinted myeloid DC recapitulate *ab initio* several characteristics of these cells, including increased CD80, CD86, PD-L1, MHC Class II, and arginase activity with decreased antigen presentation capacity (Stoitzner et al. 2008; Liu et al. 2009; Norian et al. 2009; Scarlett et al. 2012; Engelhardt et al. 2012; Tesone et al. 2013). Given that regulatory dendritic cells have been isolated from epithelial cancers prone to a microenvironmental UPR, it is possible that the tumor UPR is a key modulator of myeloid antigen presenting cell, and ultimately, T cell function. A comparison of TERS-imprinted myeloid-derived dendritic cells with TIDC in different murine experimental systems and human patients is presented in Table 18.1.

18.6 Implications of TERS-Directed Cross-Priming on Fate Determination of CD8 T Cells

Initial lineage analysis of $CD8^+$ T cells cross-primed by TERS-imprinted bone marrow derived DC showed transcriptional upregulation of the cytokines *Il-10* and *Tnf-* α but not *Il-17*, upregulation of *Foxp3*, and downregulation of the costimulatory molecule CD28. LAG3, a negative costimulatory molecule (Huard et al. 1994) found on tumor-infiltrating T cells (Grosso et al. 2007), was slightly up-regulated. When we analyzed the 96-h TERS-imprinted myeloid DC:T cell co-culture supernatant, we observed increased secretion of IL-2 but no elevation of IL-10,

Table 18.1 Comparison of different types of tumor associated myeloid cells and their effects on T cells

Type of cells	Mechanism of origin	Effect on CD8 T Cells	Mechanism of action	CTL Function	Ref.
MDSC (Gr1+)	Unknown	Inhibit naïve cell expansion	Immature DC	ND	(Kusmartsev et al. 2004)
			TCR nitration		
TuDC (CD11c+)	Unknown	Inhibit naïve cell expansion	Arginase and rescued by norvaline	ND	(Norian et al. 2009)
		Dominant suppression			
TuDC (CD11c+)	Unknown	Inhibit naïve cell expansion and Ag experienced cell re-stimulation	Ag experienced CD8 T cells rescued by TLR7/TLR9 ligands	Decreased	(Engelhardt et al. 2012)
Reg DC (MHC II/ CD11c+/CD86+)	Tumor-derived PGE2, TGF-β1	Inhibit tumor Ag experienced T cell re-stimulation	PD-L1, arginase?	ND	(Scarlett et al. 2012)
		Dominant suppression			
BMDC* (CD11c+)	Transmissible UPR	Inhibit naïve cell expansion	Reduced Ag presentation	Decreased	(Mahadevan et al. 2012)
		Dominant suppression	Arginase and rescued by norvaline		

**BMDC* bone marrow-derived dendritic cells

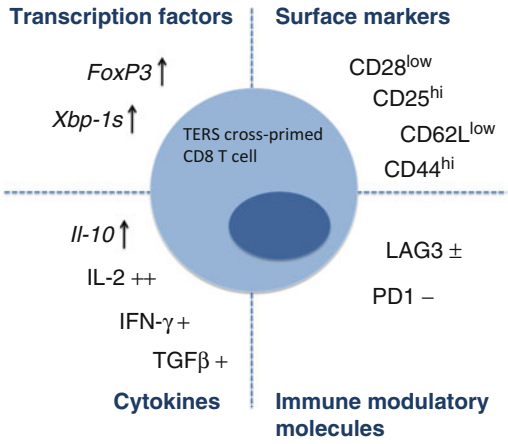

Fig. 18.3 Synopsis of phenotypic characteristics of murine CD8 T cells cross primed by TERS-imprinted myeloid DC

IL-17, IFN-γ or TNF-α compared to control (Fig. 18.3). A provisional conclusion is that CD8 T cells cross-primed by TERS-imprinted bone marrow-derived DC display an uncommitted phenotype with potential suppressive characteristics (CD28 downregulation and *Il-10* upregulation) (Filaci et al. 2007). Surprisingly, $CD8^+$ T cells cross-primed by TERS-imprinted BMDC also demonstrated disproportionately high splicing of *Xbp-1* compared to only modest upregulation of other UPR elements, the significance of which remains unknown.

In sum, the phenotype of CD8+ T cells cross-primed by TERS-imprinted myeloid DC appears similar to that of $CD8^+/CD28^-$ regulatory T cells secreting IL-10 and TNF-α, and expressing FOXP3, which have been found to infiltrate a variety of human tumors (Becker et al. 2000; Kruger et al. 2001; Filaci et al. 2007; Mahic et al. 2008). It still remains to be seen whether, like human CD8 suppressor T cells, TERS-directed CD8 T cells have suppressor functions effected by surface ecto-ATPases (e.g. CD39) and/or soluble mediators (e.g. IL-10). A comparison of the CD8+ T cell phenotype derived from TERS-imprinted APC with CD8+T cells infiltrating human tumors is presented in Table 18.2.

18.7 The Effect of TERS *In Vivo* and Mechanism of Generation

Several lines of evidence suggest that TERS is operational *in vivo*. First C57BL/6 mice injected intra-peritoneally with TERS develop an ER stress response in liver cells characterized by the up-regulation of *Grp78*, *Chop* and spliced *Xbp-1*. This suggests that a tissue that is sensitive to ER stress induction, the liver, readily becomes a target of TERS administered systemically.

Table 18.2 Comparison of tumor-associated CD8 regulatory T cells

Immune phenotype	Species	Mechanism of Origin	Inhibitory Effects	Mechanism of Action	Ref.
CD8+/ CD28-/ CD45RA+/ CCR7-/ FOXP3-/CD56-	Human	Soluble factors (IL-2, IL-10, TGF-b, GM-CSF)	Suppression of allogeneic CD8 and CD4 T cell expansion	CD39	(Fenoglio et al. 2008; Filaci et al. 2007; Parodi et al. 2013)
		Agnon-specific *in vitro*		IL-10	
CD8+/ CD45RO+/ CCR7+/ IL-10+	Human	Tumor-associated plasmacytoid DC	Suppression of Ag-specific and allogeneic T cell activation	IL-10?	(Wei et al. 2005)
CD8+/ CD28-/ FOXP3+	Mouse	TERS-imprinted myeloid DC (arginase-dependent)	Impaired CTL [a]function	ND[b]	(Mahadevan et al. 2012)

[a]*CTL* cytotoxic T lymphocytes
[b]*ND* not determined

18.7.1 TERS-Imprinted Myeloid Cells Promote Tumor Progression In Vivo

The effect of TERS on tumor growth was examined in C57BL/6 mice inoculated subcutaneously with B16.F10 tumor cells admixed with TERS-imprinted bone marrow-derived DC according to Prehn (1972). Under these conditions, we noted an earlier tumor initiation, accelerated tumor growth, and decreased survival when compared to mice receiving B16.F10 tumor cells admixed with control bone marrow-derived DC, or tumor cells alone (Mahadevan et al. 2012). Thus, bone marrow-derived DC polarized by ER-stressed tumor cells facilitate tumor growth *in vivo*. B16.F10 tumors seeded with TERS-imprinted bone marrow-derived DC contained about half the percentage of tumor infiltrating $CD8^+$ T cells as compared with control B16.F10 tumors. Interestingly, while we found a decreased number of CD8 T cells in tumors, we found no difference in the draining lymph nodes, implying the local nature of this phenomenon.

TERS-imprinted bone marrow-derived DC also function to dysregulate anti-tumor T cell immunity, allowing immune escape. For instance, TC1.OVA prostate cancer cells that constitutively express OVA, which functions as a tumor rejection antigen (Redmond et al. 2007), do not form tumors in mice reflecting their immunogenic status. However, when inoculated admixed with TERS-imprinted bone marrow-derived DC, they form transient tumors 6–10 days post-injection (Mahadevan et al. 2012).

18.7.2 *TERS Is Produced* In Vivo *During Tumor Formation*

New evidence shows that tumor-infiltrating myeloid cells *in vivo* display TERS characteristics. CD11b^{+} myeloid cells isolated from B16.F10 tumors implanted in C57BL/6 mice, or from spontaneous intestinal adenomas in *adenomatous polyposis coli* (*APC*) mice, display both an upregulation of the UPR and the mixed pro-inflammatory/suppressive phenotype typical of the TERS signature compared with bone marrow- or spleen-derived myeloid cells from tumor-bearing mice (Rodvold et al. 2014b).

18.7.3 *TERS Requires Ire1α Signaling in Transmitter Cells*

Because the ER stress response is under the control of three main sensors (IRE1α, PERK, ATF6), we began to deconvolute their precise role in the generation of TERS. Using MEFs each deficient in a single arm of the UPR, we found that compared with wild type MEFs the production of TERS was greatly diminished in IRE1α KO MEFs but not in PERK KO or ATF6 KO MEFs, providing the first indication that TERS generation may be mainly due to IRE1α signaling (Rodvold et al. 2014b). A hypothetical model of the signaling events involved in the generation of TERS is illustrated in Fig. 18.4.

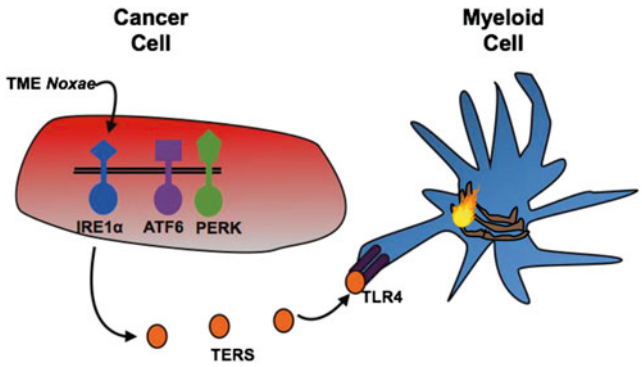

Fig. 18.4 Hypothetical model for the generation of TERS in transmitter cells. The model predicts that of the three main UPR sensors (PERK, ATF6 and IRE1α), Ire1α is responsible for the generation/release of the TERS factor(s) from stressed transmitter cancer cells. Myeloid cells (macrophages and dendritic cells) receive TERS factor(s), which is sensed in part by TLR4, and are polarized to a phenotype characterized by activation of the UPR (flame) and a pro-inflammatory/suppressive phenotype that facilitates tumor growth (see text for details)

18.8 Therapeutic Approaches Targeting the Tumor UPR

In the previous sections we discussed the cell-intrinsic role of the UPR in tumor adaptation and survival, as well as its putative cell-extrinsic role in polarizing myeloid antigen presenting cells to a phenotype that facilitates tumor outgrowth via T cell-dependent and independent mechanisms. Considering this dual role, targeting the UPR in the tumor microenvironment will likely have a dual benefit: impairing tumor cell microenvironmental adaptation and survival, and disabling a mechanism of host immune subversion. Based on our current understanding, the cellular targets, of any such intervention would be the tumor cell, myeloid antigen presenting cells, and CD8+ T cells (Fig. 18.5a). It remains to be seen whether CD4 T cell immunity is also adversely affected by the cell-extrinsic effects of the UPR.

The UPR is tumor microenvironment-specific as demonstrated by studies showing that peritumoral areas do not express UPR genes and that a constitutive UPR takes place within spontaneously growing tumors, though heterogeneously within a tumor mass (Spiotto et al. 2010). In addition, several lines of evidence indicate that UPR inhibitors selectively target tumor cells, as discussed below.

As the UPR represents an adaptive mechanism to cope with ER stress, targeting the UPR will likely take the following forms: (1) inhibition of the UPR in tumor cells with high levels of basal ER stress (eg. microenvironment-driven: hypoxia, glucose deprivation; tumor-intrinsic: secretory tumors, like myeloma), or (2) exacerbation of ER stress and consequent induction of cytotoxic/apoptotic effects. While each of these approaches will individually exploit tumor microenvironmental ER stress, either by its induction *or* by targeting its adaptive response (the UPR), they alone may not be sufficient to control the UPR within the complex and heterogeneous tumor microenvironment. For instance, exacerbating ER stress alone may exhibit cytotoxicity, especially in hypoxic/nutrient deprived areas; however, tumor cells mounting a UPR that leads to survival will have a UPR-mediated adaptive advantage, including resistance to chemotherapy (Pyrko et al. 2007) and host immunity. On the other hand, only inhibiting the UPR will target tumor cells with increased basal ER stress due to heterogenous microenvironmental *noxae*, sparing cells in more vascularized areas. We propose that optimal targeting of the UPR should take the form of inducing ER stress in tumor cells (fueling the fire) while concomitantly inhibiting the UPR (locking up the extinguisher) (Fig. 18.5b). In sum, this combinatorial mechanism will simultaneously take advantage of the cytotoxic potential of ER stress while inhibiting the response mechanism needed to adapt.

These strategies have already gained some experimental support. Bortezomib, a proteasome inhibitor that induces accumulation of proteins thus exacerbating ER stress, causes significantly higher cytotoxicity in hypoxic HeLa and human colorectal cancer cells than in normoxic cells, an effect dependent on ER protein load and consequent ER stress (Fels et al. 2008). Similarly, the induction of ER stress with a

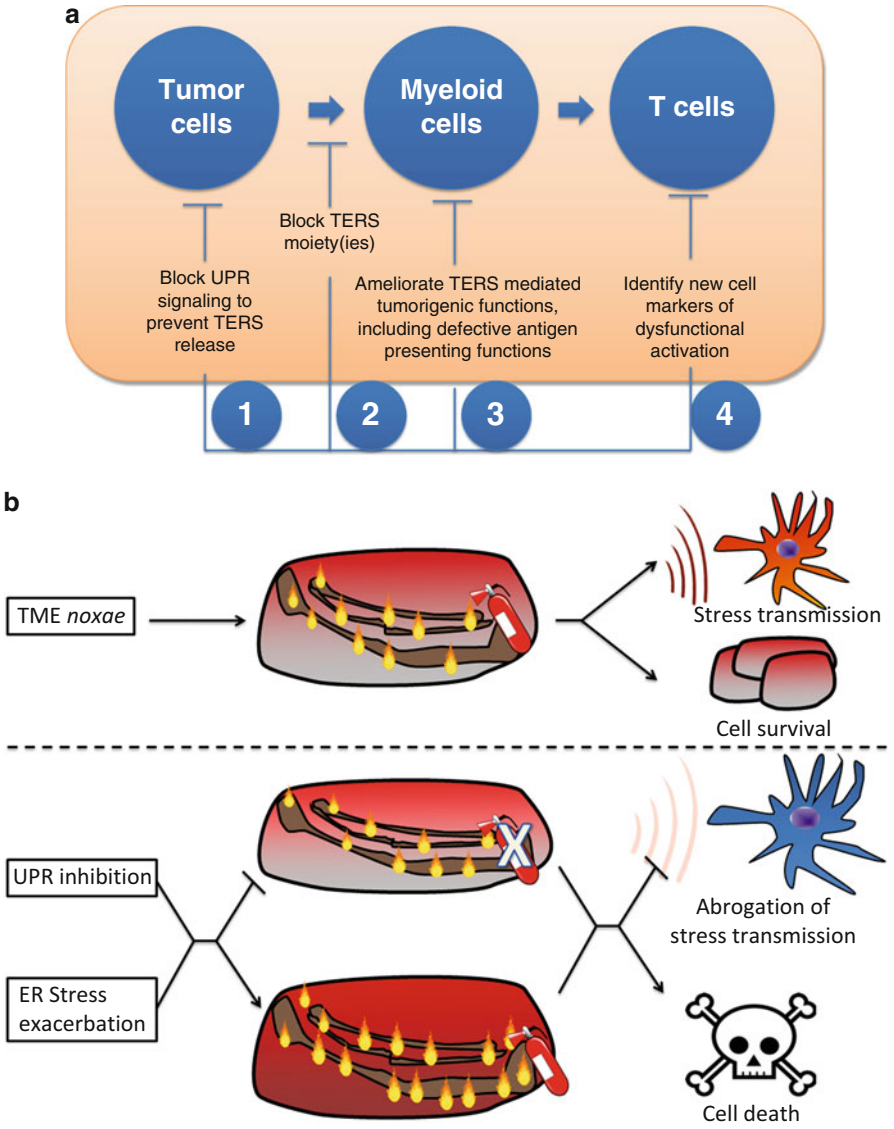

Fig. 18.5 Targeting the cell-extrinsic effects of the UPR in the tumor microenvironment. **(a)** Points of therapeutic intervention against the tumor-promoting effects of TERS. **(b)** Strategies for pharmacological control of the UPR in the context of cancer. (*upper panel*) Before any intervention, cancer cells exhibit a basal level of unfolded proteins (flames) compensated by the UPR (fire extinguisher). A combinatorial approach should involve tuning down the UPR (*middle panel*) with simultaneous exacerbation of ER stress (*lower panel*), hence inducing cell death in cells unable to cope with ER stress, as well as inhibiting the negative cell-extrinsic effects of the tumor UPR

targeted thapsigargin pro-drug, celecoxib, or bortezomib, induces glioblastoma cell death, especially in hypoxic areas of the tumor (Johnson et al. 2002; Denmeade et al. 2012; Schonthal 2013). Combination of the ER stress inducers bortezomib and celecoxib, or its non-coxib analogue, 2,5-dimethyl-celecoxib (DMC), causes severe ER stress and apoptosis in murine glioblastoma cells *in vitro* and *in vivo* (Kardosh et al. 2008).

Inhibition of *Xbp-1* splicing in multiple myeloma with the IRE1α endoribonuclease small molecule inhibitor, STF-083010, results in tumor cell-specific death *in vitro* and *in vivo* (Papandreou et al. 2011). Similarly, irestatin, a small molecule inhibitor of IRE1α endoribonuclease activity, inhibits hypoxic human myeloma and colon cancer cell survival and colony formation *in vitro*, as well as *in vivo* tumorigenesis in a heterotopic xenograft model (Papandreou et al. 2011). Targeting cell surface GRP78 in colon and lung cancer in mice with a monoclonal antibody (mAb159) causes tumor regression in vivo (Liu et al. 2013). Lastly, inhibition of GRP78 activation with active compounds present within the herbal medicine Ponciri fructis or the pyrone-type polyketide, verrucosidin, exhibits selective cytotoxicity in human pancreatic cancer cells or colon cancer cells undergoing glucose deprivation-induced ER stress (Park et al. 2007; Cha et al. 2009).

Inducing ER stress while concomitantly inhibiting the adaptive UPR has also begun to find experimental support. For instance, it has been shown that the mechanism of bortezomib's cytotoxic activity in myeloma cells is its ability to inhibit *Xbp1* splicing via stabilization of unspliced XBP-1, which acts as a dominant negative suppressor of XBP1-s, while inducing ER stress via proteasome inhibition (Lee et al. 2003a). Congruently, the induction of ER stress with bortezomib or 17-AAG in myeloma cells was shown to synergize with the activity of transgenic or small molecule-mediated inhibition of *Xbp-1* splicing resulting in the induction of greater and irreparable cytotoxicity than either agent alone *in vitro* and *in vivo* (Lee et al. 2003a; Mimura et al. 2012). In human pancreatic cancer cells, bortezomib reduces GRP78 and CHOP expression under ER stress conditions and sensitizes them to ER stress-inducing compounds, including thapsigargin, tunicamycin, and cisplatin, yielding synergistic cytotoxicity *in vitro* and *in vivo* (Nawrocki et al. 2005). GSK2606414, a small molecule inhibitor of PERK autophophorylation and downstream eIF2α phosphorylation, cooperates with ER stress induced by hypoxia or thapsigargin, causing greater inhibition of *in vitro* clonogenic survival of pancreatic and colon cancer cells than either PERK inhibition or ER stress induction alone (Axten et al. 2012; Cojocari et al. 2013). Epigallocatechin gallate, which inhibits GRP78 by targeting its ATP-binding domain, sensitizes human glioma cells to ER stress induced by the chemotherapeutic agent, temozolomide, resulting in synergistic cyotoxicity, greater than either agent alone (Pyrko et al. 2007). There are several chemical UPR inhibitors that have displayed efficacy against tumor growth *in vitro* and *in vivo* (reviewed in (Li et al. 2011) and (Schonthal 2013)) these are presented in Tables 18.3 and 18.4.

While there has been recent interest in developing UPR inhibitors active against tumor cells, there has been little or no investigation the effect of tumor UPR inhibition on the host anti-tumor immune response. A link between the tumor UPR and the immune response was originally suggested by the finding that silencing of *Grp78* in

Table 18.3 Tumor cell active ER stress/UPR modulators

Compound	Mechanism of action	Tumor cell type	Reference
Thapsigargin, celecoxib	ER stress induction via SERCA inhibition	Breast, prostate	(Denmeade et al. 2012; Johnson et al. 2002; Schonthal 2013)
Biguanides, versipelostatin, pyruvinium pamoate	Inhibition of GRP78, ATF6, Xbp1 response to glucose deprivation	Fibrosarcoma, HeLa	(Saito et al. 2009)
Verrucosidin, Epigallocatechin, *Ponciri fructis* active compound	Inhibition of GRP78	Breast, glioblastoma	(Cha et al. 2009; Park et al. 2007; Pyrko et al. 2007)
STF-083010, MKC-3946	IRE1 endoribonuclease inhibition	Multiple myeloma	(Papandreou et al. 2011); (Mimura et al. 2012)
Irestatin	IRE1 endoribonuclease inhibition	Multiple myeloma, colon	(Papandreou et al. 2011)
Bortezomib	IRE1 dominant negative inhibition	Multiple myeloma	(Lee et al. 2003a; Mimura et al. 2012)
	ER stress induction via proteasome inhibition		
GSK2606414	PERK kinase inhibition	Pancreas, colorectal	(Axten et al. 2012; Cojocari et al. 2013)

Table 18.4 Cell-nonautonomous control of cellular stress responses

Stress response system	Organism	Transmitter (effectors)	Receiver (sensors)	Soluble mediators	Reference
HSR	C. elegans	Thermosensory neuron (ND)	Muscle cell (ND)	ND (unc31-mediated)	(Prahlad and Morimoto 2011)
HSR	C. elegans	Motor neuron (NT)	Muscle cell (postsynaptic NT receptor)	ACh, GABA	(Garcia et al. 2007)
HSR	C. elegans	Muscle, intestinal cell (pha-4)	intestinal, pharyngeal, excretory cells (pha-4)	ND (unc31/unc13-independent)	(van Oosten-Hawle et al. 2013)
UPR^{MT}	C. elegans	Neuron (ND)	Intestinal cell (ND)	ND	(Durieux et al. 2011)
UPR^{ER}	C. elegans	Neuron (ND)	Multiple somatic tissues (ND)	ND	(Sun et al. 2012)
UPR^{ER}	C. elegans	Neuron (xbp1)	Intestinal cell (xbp1)	ND (unc13-mediated)	(Taylor and Dillin 2013)
UPR^{ER}	Mouse	Epithelial tumor cell (IRE1)	Macrophage	ND	(Mahadevan et al. 2012; Mahadevan et al. 2011b; Rodvold et al. 2014a)
	Human		Myeloid dendritic cell		
			Tumor cell (TLR4, IRE1)		

mouse fibrosarcoma cells inhibited growth in an *in vivo* syngeneic transplantation model due, in part, to increased tumor cell-specific memory T cell generation (Jamora et al. 1996). In addition, overexpression of GRP78 in murine insulinoma cells leads to impaired CD8 T cell priming and inhibition of killing, when GRP78-overexpressing tumor cells were used to prime cytotoxic T cell lines, as targets, respectively (Wang et al. 2007). Discovery and characterization of the effect of TERS on host immunity has continued this line of inquiry (Zanetti 2013).

Based on our findings on transmissible ER stress, it appears that a fruitful avenue for therapeutic development will be to develop decoy systems (antibodies, aptamers, etc.) to intercept the TERS factor(s) in the extracellular space (Fig. 18.5a). In this scenario neutralization of TERS would also inhibit the polarization of myeloid cells to a pro-inflammatory/suppressive phenotype, and in turn prevent and unfetter the untoward effects on T cell-mediated immunity, perhaps permitting more effective autochthonous or vaccine-induced anti-tumor immune responses. In addition, TERS may induce tumor-infiltrating myeloid cells to produce tumorigenic cytokines and adversely affects antigen presentation (see Sects. 3.2 and 3.3 for discussion). Lastly, as the downstream effects of TERS on T cell priming are elucidated (i.e. polarization toward a suppressive phenotype), new targets for therapy will come to light (e.g., ecto-ATPases, immune checkpoint molecules, UPR signaling components). Targeting the tumor-infiltrating myeloid cell UPR, the tumor cell UPR, and ultimately suppressive T cells, will reset the multifaceted dysregulation of the tumor microenvironment that hinders anti-tumor immunity.

18.9 Conclusions and Perspectives

During their growth, tumor cells are subjected to *noxae* that exist in the tumor microenvironment and are able to induce the ER stress response that, as discussed, leads to multifaceted dyregulation. Some of them are cell-intrinsic in nature and promote tumor cell adaptation and survival. Others are cell-extrinsic and affect the function of neighboring cells—immune cells, cancer cell themselves (Rodvold et al. 2014a), and stromal cells in a significant way. The intent of this chapter was to bring attention to effects on immune cells.

A hitherto unappreciated phenomenon, transmissible ER stress (TERS), seems to link together tumor cells and immune cells. Receiver myeloid cells, macrophages and dendritic cells become polarized, via ER stress transmission, to a mixed, pro-inflammatory/suppressive phenotype. The pro-inflammatory component, an exquisite innate immune trait, is per se sufficient to fuel tumor growth through a variety of mechanisms including perhaps increasing tumor cell proliferation, survival, and the mutational rate (Grivennikov et al. 2010; Tili et al. 2011). Importantly, however, we found that a mixed, pro-inflammatory/suppressive phenotype also hampers essential immunological functions of dendritic cells, i.e., antigen presentation and T cell priming, hence disabling the adaptive T cell response (Mahadevan and Zanetti 2011; Zanetti 2013). Combined, these effects favor faster tumor growth (Fig. 18.6).

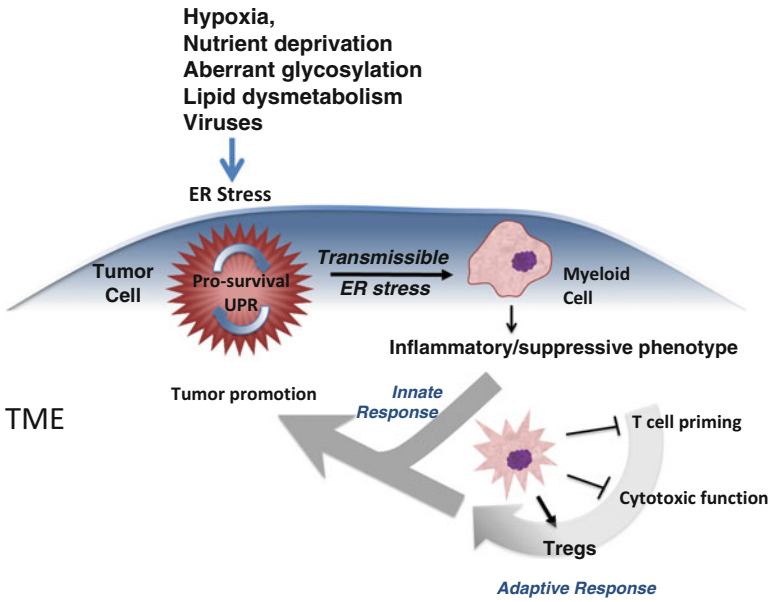

Fig. 18.6 Cell non-autonomous regulation of immunity within the tumor microenvironment via transmissible ER stress. Various *noxae* in the tumor microenvironment (*TME*) perturb tumor cells inducing a UPR. Under the umbrella of the UPR response (*blue shade*) there occur multiple events, which ultimately influence neighboring myeloid cells and T cells. In this framework, the tumor UPR acts as a cell-intrinsic tumor pro-survival factor (*circular arrow*). ER-stressed tumor cells also release many factors (cytokines, chemokines metalloproteinases etc.) among which is a factor(s) able to polarize myeloid cells by transmitting ER stress to them (*second hand stress*). As a result receiver myeloid cells undergo a UPR, and acquire an inflammatory/suppressive phenotype that facilitates tumor growth directly (innate response) and/or via dysregulation of T cell immunity (adaptive response). Together, these T cell-independent and –dependent effects (*merging arrows*) favor tumor growth (Adapted from Mahadevan and Zanetti (2011))

That ER stress can be transmitted from tumor cells to myeloid cells is a new facet in the complex intercellular interplay of the tumor microenvironment. A group of cancer cells (those experiencing a ER stress response) influencing the community of neighboring cells is reminiscent of *quorum sensing* in bacteria (Miller and Bassler 2001). Similar to the growth advantage conferred to bacteria by *quorum sensing*, the transmission of ER stress empowers a group of cancer cells to control the tumor microenvironment and initiate a cascade of pro-tumorigenic events. This new mechanism of immune subversion could explain, at least in part, why autochthonous or vaccine-induced anti-cancer T cell responses are ineffective at controlling tumor growth.

Cell non-autonomous control of several protein-folding homeostatic systems has been recently identified. These include the heat shock response (HSR), the mitochondrial UPR (UPR^{mt}), and the endoplasmic UPR (UPR^{ER}) in the nematode, *Caenorhabditis elegans*. In each of these systems, proteotoxic stress adaptation signaling in one cell (e.g. neuron, muscle cell) is sensed in a distal cell (e.g. intestinal cell), which

upregulates its own proteotoxic stress adaptation machinery, leading to cellular adaptation, resistance to death, and in some cases, organismal longevity. No soluble factors mediating this cell non-autonomous stress signaling have yet been identified, though it is suggested that neuron-derived vesicular trafficking is involved in some, but not all, systems of cell non-autonomous control of proteotoxic stress (reviewed in (Taylor and Dillin 2013)). It is possible that TERS-based tumor-myeloid cell communication is an evolutionary descendant of these more ancient intercellular communication networks, and implies that other cellular stress-based signaling may occur in humans, especially in disease states characterized by pathogenic proteostasis and inflammation (e.g. Type I diabetes and neurodegenerative disease).

In conclusion, the cell extrinsic effects of the tumor UPR represent a new variable in the complex and still poorly-understood interplay between cancer and the immune system. We have presented some general principles for interfering with the UPR within the tumor microenvironment. In our opinion, two important practical considerations can be made. One is that by interfering with the UPR in cancer cells one may succeed in causing cell death and also prevent the initiation of transmissible ER stress. The other is to intercept the transmissible ER stress factor(s) or block the effects of transmissible ER stress on receiver myeloid cells. Overall, controlling the UPR within the tumor microenvironment may represent an opportunity to complement conventional therapies and immunotherapy in the future.

Acknowledgments This work was supported in part by a grant from the UCSD Academic Senate.

References

Akers JC, Gonda D, Kim R, Carter BS, Chen CC (2013) Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol 113:1–11

Arap MA, Lahdenranta J, Mintz PJ, Hajitou A, Sarkis AS, Arap W, Pasqualini R (2004) Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. Cancer Cell 6:275–284

Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, Li WH, Heerding DA, Minthorn E, Mencken T et al (2012) Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem 55:7193–7207

Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357:539–545

Balkwill F, Charles KA, Mantovani A (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7:211–217

Bartoszewski R, Brewer JW, Rab A, Crossman DK, Bartoszewska S, Kapoor N, Fuller C, Collawn JF, Bebok Z (2011) The unfolded protein response (UPR)-activated transcription factor X-box-binding protein 1 (XBP1) induces microRNA-346 expression that targets the human antigen peptide transporter 1 (TAP1) mRNA and governs immune regulatory genes. J Biol Chem 286:41862–41870

Becker JC, Vetter CS, Schrama D, Brocker EB, Thor Straten P (2000) Differential expression of CD28 and CD94/NKG2 on T cells with identical TCR beta variable regions in primary melanoma and sentinel lymph node. Eur J Immunol 30:3699–3706

Bell D, Chomarat P, Broyles D, Netto G, Harb GM, Lebecque S, Valladeau J, Davoust J, Palucka KA, Banchereau J (1999) In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. J Exp Med 190:1417–1426

Beverly B, Kang SM, Lenardo MJ, Schwartz RH (1992) Reversal of in vitro T cell clonal anergy by IL-2 stimulation. Int Immunol 4:661–671

Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J et al (2005) ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. EMBO J 24:3470–3481

Blais JD, Addison CL, Edge R, Falls T, Zhao H, Wary K, Koumenis C, Harding HP, Ron D, Holcik M, Bell JC (2006) Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol Cell Biol 26:9517–9532

Bonizzi G, Karin M (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25:280–288

Bronte V, Zanovello P (2005) Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 5:641–654

Burnet FM (1970) The concept of immunological surveillance. Prog Exp Tumor Res 13:1–27

Burnet FM (1971) Immunological surveillance in neoplasia. Transplant Rev 7:3–25

Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11:85–95

Cavanagh LL, Bonasio R, Mazo IB, Halin C, Cheng G, van der Velden AW, Cariappa A, Chase C, Russell P, Starnbach MN et al (2005) Activation of bone marrow-resident memory T cells by circulating, antigen-bearing dendritic cells. Nat Immunol 6:1029–1037

Cha MR, Yoon MY, Son ES, Park HR (2009) Selective cytotoxicity of Ponciri Fructus against glucose-deprived PANC-1 human pancreatic cancer cells via blocking activation of GRP78. Biosci Biotechnol Biochem 73:2167–2171

Chaffer CL, Weinberg RA (2011) A perspective on cancer cell metastasis. Science 331:1559–1564

Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, Banerjee A, Longworth SA, Vinup KE, Mrass P, Oliaro J et al (2007) Asymmetric T lymphocyte division in the initiation of adaptive immune responses. Science 315:1687–1691

Chaux P, Favre N, Martin M, Martin F (1997) Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression in rats. Int J Cancer J Int Du Cancer 72:619–624

Chen P, Bonaldo P (2013) Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. Int Rev Cell Mol Biol 301:1–35

Chen L, Jarujaron S, Wu X, Sun L, Zha W, Liang G, Wang X, Gurley EC, Studer EJ, Hylemon PB 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

Cojocari D, Vellanki RN, Sit B, Uehling D, Koritzinsky M, Wouters BG (2013) New small molecule inhibitors of UPR activation demonstrate that PERK, but not IRE1alpha signaling is essential for promoting adaptation and survival to hypoxia. Radiother Oncol 108:541–547

Coussens LM, Zitvogel L, Palucka AK (2013) Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science 339:286–291

Cullen SJ, Fatemie S, Ladiges W (2013) Breast tumor cells primed by endoplasmic reticulum stress remodel macrophage phenotype. Am J Cancer Res 3:196–210

Dai F, Liu L, Che G, Yu N, Pu Q, Zhang S, Ma J, Ma L, You Z (2010) The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. BMC Cancer 10:220

Daneshmand S, Quek ML, Lin E, Lee C, Cote RJ, Hawes D, Cai J, Groshen S, Lieskovsky G, Skinner DG et al (2007) Glucose-regulated protein GRP78 is up-regulated in prostate cancer and correlates with recurrence and survival. Hum Pathol 38:1547–1552

Davidson DJ, Haskell C, Majest S, Kherzai A, Egan DA, Walter KA, Schneider A, Gubbins EF, Solomon L, Chen Z et al (2005) Kringle 5 of human plasminogen induces apoptosis of

endothelial and tumor cells through surface-expressed glucose-regulated protein 78. Cancer Res 65:4663–4672

Davoli T, de Lange T (2011) The causes and consequences of polyploidy in normal development and cancer. Annu Rev Cell Dev Biol 27:585–610

de Almeida SF, Fleming JV, Azevedo JE, Carmo-Fonseca M, de Sousa M (2007) Stimulation of an unfolded protein response impairs MHC class I expression. J Immunol 178:3612–3619

DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA (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

Denardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA et al (2011) Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 1:54–67

Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, Harding HP, Ron D (2004) Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol 24:10161–10168

Denmeade SR, Mhaka AM, Rosen DM, Brennen WN, Dalrymple S, Dach I, Olesen C, Gurel B, Demarzo AM, Wilding G et al (2012) Engineering a prostate-specific membrane antigen-activated tumor endothelial cell prodrug for cancer therapy. Sci Transl Med 4:140ra186

Dong D, Ni M, Li J, Xiong S, Ye W, Virrey JJ, Mao C, Ye R, Wang M, Pen L 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

Drogat B, Auguste P, Nguyen DT, Bouchecareilh M, Pineau R, Nalbantoglu J, Kaufman RJ, Chevet E, Bikfalvi A, Moenner M (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

Durieux J, Wolff S, Dillin A (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 144:79–91

Engelhardt JJ, Boldajipour B, Beemiller P, Pandurangi P, Sorensen C, Werb Z, Egeblad M, Krummel MF (2012) Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. Cancer Cell 21:402–417

Fels DR, Ye J, Segan AT, Kridel SJ, Spiotto M, Olson M, Koong AC, Koumenis C (2008) Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. Cancer Res 68:9323–9330

Fenoglio D, Ferrera F, Fravega M, Balestra P, Battaglia F, Proietti M, Andrei C, Olive D, Antonio LC, Indiveri F, Filaci G (2008) Advancements on phenotypic and functional characterization of non-antigen-specific CD8+CD28- regulatory T cells. Hum Immunol 69:745–750

Fernandez PM, Tabbara SO, Jacobs LK, Manning FC, Tsangaris TN, Schwartz AM, Kennedy KA, Patierno SR (2000) Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. Breast Cancer Res Treat 59:15–26

Filaci G, Fravega M, Setti M, Traverso P, Millo E, Fenoglio D, Negrini S, Ferrera F, Romagnoli A, Basso M et al (2006) Frequency of telomerase-specific CD8+ T lymphocytes in cancer patients. Blood 107:1505–1512

Filaci G, Fenoglio D, Fravega M, Ansaldo G, Borgonovo G, Traverso P, Villaggio B, Ferrera A, Kunkl A, Rizzi M et al (2007) CD8+ CD28- T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. J Immunol 179:4323–4334

Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC et al (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192:1027–1034

Friese MA, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A, Weller M (2004) RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res 64:7596–7603

Fu Y, Li J, Lee AS (2007) GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res 67:3734–3740
Fu Y, Wey S, Wang M, Ye R, Liao CP, Roy-Burman P, Lee AS (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 U S A 105:19444–19449
Gabrilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP (1997) Decreased antigen presentation by dendritic cells in patients with breast cancer. Clin Cancer Res 3:483–490
Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12:253–268
Garcia SM, Casanueva MO, Silva MC, Amaral MD, Morimoto RI (2007) Neuronal signaling modulates protein homeostasis in Caenorhabditis elegans post-synaptic muscle cells. Genes Dev 21:3006–3016
Goodall JC, Wu C, Zhang Y, McNeill L, Ellis L, Saudek V, Gaston JS (2010) Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. Proc Natl Acad Sci U S A 107:17698–17703
Granados DP, Tanguay PL, Hardy MP, Caron E, de Verteuil D, Meloche S, Perreault C (2009) ER stress affects processing of MHC class I-associated peptides. BMC Immunol 10:10
Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118:285–296
Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M (2009) IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 15:103–113
Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899
Grosso JF, Kelleher CC, Harris TJ, Maris CH, Hipkiss EL, De Marzo A, Anders R, Netto G, Getnet D, Bruno TC et al (2007) LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. J Clin Invest 117:3383–3392
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
He B (2006) Viruses, endoplasmic reticulum stress, and interferon responses. Cell Death Differ 13:393–403
Heazlewood CK, Cook MC, Eri R, Price GR, Tauro SB, Taupin D, Thornton DJ, Png CW, Crockford TL, Cornall RJ et al (2008) Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. PLoS Med 5:e54
Howarth M, Williams A, Tolstrup AB, Elliott T (2004) Tapasin enhances MHC class I peptide presentation according to peptide half-life. Proc Natl Acad Sci U S A 101:11737–11742
Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH (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
Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, Divino CM, Chen SH (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
Huard B, Gaulard P, Faure F, Hercend T, Triebel F (1994) Cellular expression and tissue distribution of the human LAG-3-encoded protein, an MHC class II ligand. Immunogenetics 39:213–217
Jacobs JF, Nierkens S, Figdor CG, de Vries IJ, Adema GJ (2012) Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? Lancet Oncol 13:e32–e42
Jamora C, Dennert G, Lee AS (1996) Inhibition of tumor progression by suppression of stress protein GRP78/BiP induction in fibrosarcoma B/C10ME. Proc Natl Acad Sci U S A 93:7690–7694
Jiang HY, Wek SA, McGrath BC, Scheuner D, Kaufman RJ, Cavener DR, Wek RC (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
Johnson AJ, Hsu AL, Lin HP, Song X, Chen CS (2002) The cyclo-oxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum Ca2+-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks. Biochem J 366:831–837

Kardosh A, Golden EB, Pyrko P, Uddin J, Hofman FM, Chen TC, Louie SG, Petasis NA, Schonthal AH (2008) Aggravated endoplasmic reticulum stress as a basis for enhanced glioblastoma cell killing by bortezomib in combination with celecoxib or its non-coxib analogue, 2,5-dimethyl-celecoxib. Cancer Res 68:843–851

Kawai O, Ishii G, Kubota K, Murata Y, Naito Y, Mizuno T, Aokage K, Saijo N, Nishiwaki Y, Gemma A et al (2008) Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 113:1387–1395

Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M (2009) Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature 457:102–106

Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, Smyth MJ, Schreiber RD (2007) Adaptive immunity maintains occult cancer in an equilibrium state. Nature 450:903–907

Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, Drake C, Pardoll D, Yu H (2009) Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell 15:114–123

Kruger K, Buning C, Schriever F (2001) Activated T lymphocytes bind in situ to stromal tissue of colon carcinoma but lack adhesion to tumor cells. Eur J Immunol 31:138–145

Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H (2008) Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. J Clin Invest 118:3367–3377

Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI (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

Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M (2006) IL-23 promotes tumour incidence and growth. Nature 442:461–465

Lee AH, Iwakoshi NN, Anderson KC, Glimcher LH (2003a) Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. Proc Natl Acad Sci U S A 100:9946–9951

Lee AH, Iwakoshi NN, Glimcher LH (2003b) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 23:7448–7459

Li X, Zhang K, Li Z (2011) Unfolded protein response in cancer: the physician's perspective. J Hematol Oncol 4:8

Liu Q, Zhang C, Sun A, Zheng Y, Wang L, Cao X (2009) Tumor-educated CD11bhighIalow regulatory dendritic cells suppress T cell response through arginase I. J Immunol 182:6207–6216

Mahadevan NR, Zanetti M (2011) Tumor stress inside out: Cell-extrinsic effects of the unfolded protein response in tumor cells modulate the immunological landscape of the tumor microenvironment. J Immunol 187:4403–4409

Mahadevan NR, Fernandez A, Rodvold J, Almanza G, Zanetti M (2010) Prostate cells undergoing ER stress in vitro and in vivo activate transcription of pro-inflammatory cytokines. J Inflam Res 3:99–103

Mahadevan NR, Rodvold J, Almanza G, Fernandez Perez A, Wheeler MC, Zanetti M (2011a) ER stress activates Lipocalin 2 transcription in prostate cancer cells in an NF-kB-dependent manner. BMC Cancer 11:229

Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF, Zanetti M (2011b) Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. Proc Natl Acad Sci U S A 108:6561–6566

Mahadevan NR, Anufreichik V, Rodvold JJ, Chiu KT, Sepulveda H, Zanetti M (2012) Cell-extrinsic effects of tumor ER stress imprint myeloid dendritic cells and impair CD8(+) T cell priming. PLoS One 7:e51845

Mahadevan NR, Rodvold JJ, Zanetti M (2013) A Janus-faced role of the unfolded protein response in antitumor immunity. Oncoimmunology 2:e23901

Mahic M, Henjum K, Yaqub S, Bjornbeth BA, Torgersen KM, Tasken K, Aandahl EM (2008) Generation of highly suppressive adaptive CD8(+)CD25(+)FOXP3(+) regulatory T cells by continuous antigen stimulation. Eur J Immunol 38:640–646

Mantovani A, Sozzani S, Locati M, Allavena P, Sica A (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 23:549–555

Mao Y, Poschke I, Kiessling R (2014) Tumour-induced immune suppression: role of inflammatory mediators released by myelomonocytic cells. J Intern Med 276:154–170

Martinon F, Chen X, Lee AH, Glimcher LH (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat Immunol 11:411–418

Massague J (2008) TGFbeta in cancer. Cell 134:215–230

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H et al (2012) Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. Blood 119:5772–5781

Misra UK, Deedwania R, Pizzo SV (2006) Activation and cross-talk between Akt, NF-kappaB, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. J Biol Chem 281:13694–13707

Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE, Davis MM (2000) Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med 6:1018–1023

Monsurro V, Nagorsen D, Wang E, Provenzano M, Dudley ME, Rosenberg SA, Marincola FM (2002) Functional heterogeneity of vaccine-induced CD8(+) T cells. J Immunol 168:5933–5942

Monsurro VV, Wang E, Yamano Y, Migueles SA, Panelli MC, Smith K, Nagorsen D, Connors M, Jacobson S, Marincola FM (2004) Quiescent phenotype of tumor-specific CD8+ T cells following immunization. Blood 104:1970–1978

Morales C, Rachidi S, Hong F, Sun S, Ouyang X, Wallace C, Zhang Y, Garret-Mayer E, Wu J, Liu B, Li Z (2014) Immune chaperone gp96 drives the contributions of macrophages to inflammatory colon tumorigenesis. Cancer Res 74:446–459

Mougiakakos D, Choudhury A, Lladser A, Kiessling R, Johansson CC (2010) Regulatory T cells in cancer. Adv Cancer Res 107:57–117

Mumm JB, Oft M (2008) Cytokine-based transformation of immune surveillance into tumor-promoting inflammation. Oncogene 27:5913–5919

Nagorsen D, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U (2003) Natural T cell immunity against cancer. Clin Cancer Res 9:4296–4303

Naugler WE, Karin M (2008) The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14:109–119

Nawrocki ST, Carew JS, Pino MS, Highshaw RA, Dunner K Jr, Huang P, Abbruzzese JL, McConkey DJ (2005) Bortezomib sensitizes pancreatic cancer cells to endoplasmic reticulum stress-mediated apoptosis. Cancer Res 65:11658–11666

Norian LA, Rodriguez PC, O'Mara LA, Zabaleta J, Ochoa AC, Cella M, Allen PM (2009) Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. Cancer Res 69:3086–3094

Ohyama S, Yonemura Y, Miyazaki I (1990) Prognostic value of S-phase fraction and DNA ploidy studied with in vivo administration of bromodeoxyuridine on human gastric cancers. Cancer 65:116–121

Osorio F, Tavernier SJ, Hoffmann E, Saeys Y, Martens L, Vetters J, Delrue I, De Rycke R, Parthoens E, Pouliot P et al (2014) The unfolded-protein-response sensor IRE-1alpha regulates the function of CD8alpha(+) dendritic cells. Nat Immunol 15:248–257

Ostrand-Rosenberg S, Sinha P (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol 182:4499–4506

Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T (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 U S A 93:13119–13124

Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M, Koong AC (2011) Identification of an Ire1alpha

endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood 117:1311–1314

Park HR, Ryoo IJ, Choo SJ, Hwang JH, Kim JY, Cha MR, Shin-Ya K, Yoo ID (2007) Glucose-deprived HT-29 human colon carcinoma cells are sensitive to verrucosidin as a GRP78 down-regulator. Toxicology 229:253–261

Parodi A, Battaglia F, Kalli F, Ferrera F, Conteduca G, Tardito S, Stringara S, Ivaldi F, Negrini S, Borgonovo G et al (2013) CD39 is highly involved in mediating the suppression activity of tumor-infiltrating CD8+ T regulatory lymphocytes. Cancer Immunol Immunother 62:851–862

Pellicciotta I, Cortez-Gonzalez X, Sasik R, Reiter Y, Hardiman G, Langlade-Demoyen P, Zanetti M (2008) Presentation of telomerase reverse transcriptase, a self-tumor antigen, is down-regulated by histone deacetylase inhibition. Cancer Res 68:8085–8093

Pickup M, Novitskiy S, Moses HL (2013) The roles of TGFbeta in the tumour microenvironment. Nat Rev Cancer 13:788–799

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431:461–466

Pinzon-Charry A, Maxwell T, Lopez JA (2005) Dendritic cell dysfunction in cancer: a mechanism for immunosuppression. Immunol Cell Biol 83:451–461

Prahlad V, Morimoto RI (2011) Neuronal circuitry regulates the response of Caenorhabditis elegans to misfolded proteins. Proc Natl Acad Sci U S A 108:14204–14209

Prehn RT (1972) The immune reaction as a stimulator of tumor growth. Science 176:170–171

Probst HC, Lagnel J, Kollias G, van den Broek M (2003) Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. Immunity 18:713–720

Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS (2007) The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res 67:9809–9816

Ranganathan AC, Zhang L, Adam AP, Aguirre-Ghiso JA (2006) Functional coupling of p38-induced up-regulation of BiP and activation of RNA-dependent protein kinase-like endoplasmic reticulum kinase to drug resistance of dormant carcinoma cells. Cancer Res 66:1702–1711

Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS (2003) Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem 278:20915–20924

Redmond WL, Gough MJ, Charbonneau B, Ratliff TL, Weinberg AD (2007) Defects in the acquisition of CD8 T cell effector function after priming with tumor or soluble antigen can be overcome by the addition of an OX40 agonist. J Immunol 179:7244–7253

Rodvold JJ, Chiu KT, Graf R, Mahadevan NR, Stupack D, Zanetti M (2014a) Transmissible ER stress licenses prostate cancer cells for pro-survival and resistance to chemotherapy. Submitted 2014

Rodvold JJ, Hiramatsu N, Mahadevan NR, Chiu KT, Niwa M, Lin J, Zanetti M (2014b) Tumor ER stress transmission to myeloid cells requires Ire1alpha. (In preparation)

Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, Mori K, Glimcher LH, Denko NC, Giaccia AJ et al (2004) XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Res 64:5943–5947

Romero-Ramirez L, Cao H, Regalado MP, Kambham N, Siemann D, Kim JJ, Le QT, Koong AC (2009) X box-binding protein 1 regulates angiogenesis in human pancreatic adenocarcinomas. Transl Oncol 2:31–38

Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM (2012) Leukocyte composition of human breast cancer. Proc Natl Acad Sci U S A 109:2796–2801

Saito S, Furuno A, Sakurai J, Sakamoto A, Park HR, Shin-Ya K, Tsuruo T, Tomida A (2009) Chemical genomics identifies the unfolded protein response as a target for selective cancer cell killing during glucose deprivation. Cancer Res 69:4225–4234

Sakaguchi S (2003) Control of immune responses by naturally arising CD4+ regulatory T cells that express toll-like receptors. J Exp Med 197:397–401
Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133:775–787
Savage PA, Vosseller K, Kang C, Larimore K, Riedel E, Wojnoonski K, Jungbluth AA, Allison JP (2008) Recognition of a ubiquitous self antigen by prostate cancer-infiltrating CD8+ T lymphocytes. Science 319:215–220
Scarlett UK, Rutkowski MR, Rauwerdink AM, Fields J, Escovar-Fadul X, Baird J, Cubillos-Ruiz JR, Jacobs AC, Gonzalez JL, Weaver J et al (2012) Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. J Exp Med 209:495–506
Schietinger A, Delrow JJ, Basom RS, Blattman JN, Greenberg PD (2012) Rescued tolerant CD8 T cells are preprogrammed to reestablish the tolerant state. Science 335:723–727
Schonthal AH (2013) Pharmacological targeting of endoplasmic reticulum stress signaling in cancer. Biochem Pharmacol 85:653–666
Schreiber RD, Old LJ, Smyth MJ (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 331:1565–1570
Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, Galluzzi L, Adjemian S, Kepp O, Niso-Santano M et al (2012) An immunosurveillance mechanism controls cancer cell ploidy. Science 337:1678–1684
Serafini P, Borrello I, Bronte V (2006) Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol 16:53–65
Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, Fuh G, Gerber HP, Ferrara N (2007) Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. Nat Biotechnol 25:911–920
Shuda M, Kondoh N, Imazeki N, Tanaka K, Okada T, Mori K, Hada A, Arai M, Wakatsuki T, Matsubara O 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
Silverstein AM (1989) Transplanatation and immunogenetics. Academic Press Inc, San Diego
Spiotto MT, Banh A, Papandreou I, Cao H, Galvez MG, Gurtner GC, Denko NC, Le QT, Koong AC (2010) Imaging the unfolded protein response in primary tumors reveals microenvironments with metabolic variations that predict tumor growth. Cancer Res 70:78–88
Steinman RM, Nussenzweig MC (2002) Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. Proc Natl Acad Sci U S A 99:351–358
Steinman RM, Hawiger D, Nussenzweig MC (2003) Tolerogenic dendritic cells. Annu Rev Immunol 21:685–711
Stoitzner P, Green LK, Jung JY, Price KM, Atarea H, Kivell B, Ronchese F (2008) Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. Cancer Immunol Immunother 57:1665–1673
Sun J, Liu Y, Aballay A (2012) Organismal regulation of XBP-1-mediated unfolded protein response during development and immune activation. EMBO Rep 13:855–860
Taylor RC, Dillin A (2013) XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell 153:1435–1447
Tesone AJ, Svoronos N, Allegrezza MJ, Conejo-Garcia JR (2013) Pathological mobilization and activities of dendritic cells in tumor-bearing hosts: challenges and opportunities for immunotherapy of cancer. Front Immunol 4:435
Thomas DA, Massague J (2005) TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 8:369–380
Tili E, Michaille JJ, Wernicke D, Alder H, Costinean S, Volinia S, Croce CM (2011) Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. Proc Natl Acad Sci U S A 108:4908–4913
Tlsty TD, Coussens LM (2006) Tumor stroma and regulation of cancer development. Annu Rev Pathol 1:119–150

Uramoto H, Sugio K, Oyama T, Nakata S, Ono K, Yoshimastu T, Morita M, Yasumoto K (2005) Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance. Lung Cancer 49:55–62

Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, Raes G, De Baetselier P (2006) Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiology 211:487–501

van Oosten-Hawle P, Porter RS, Morimoto RI (2013) Regulation of organismal proteostasis by transcellular chaperone signaling. Cell 153:1366–1378

Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW (2013) Cancer genome landscapes. Science 339:1546–1558

von Scheidt B, Leung PS, Yong MC, Zhang Y, Towne JE, Smyth MJ, Teng MW (2014) Combined anti-CD40 and anti-IL-23 monoclonal antibody therapy effectively suppresses tumor growth and metastases. Cancer Res 74:2412–2421

Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334:1081–1086

Wang M, Wang P, Liu YQ, Peng JL, Zhao XP, Wu S, He FR, Wen X, Li Y, Shen GX (2007) The immunosuppressive and protective ability of glucose-regulated protein 78 for improvement of alloimmunity in beta cell transplantation. Clin Exp Immunol 150:546–552

Warburg O (1956) On the origin of cancer cells. Science 123:309–314

Wei CH, Trenney R, Sanchez-Alavez M, Marquardt K, Woodland DL, Henriksen SJ, Sherman LA (2005) Tissue-resident memory CD8+ T cells can be deleted by soluble, but not cross-presented antigen. J Immunol 175:6615–6623

Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P (2005) Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J Clin Oncol 23:8959–8967

Wheeler MC, Rizzi M, Sasik R, Almanza G, Hardiman G, Zanetti M (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

Whiteside TL (2012) What are regulatory T cells (Treg) regulating in cancer and why? Semin Cancer Biol 22:327–334

Willimsky G, Blankenstein T (2005) Sporadic immunogenic tumours avoid destruction by inducing T-cell tolerance. Nature 437:141–146

Xing X, Lai M, Wang Y, Xu E, Huang Q (2006) Overexpression of glucose-regulated protein 78 in colon cancer. Clin Chim Acta 364:308–315

Yamazaki H, Hiramatsu N, Hayakawa K, Tagawa Y, Okamura M, Ogata R, Huang T, Nakajima S, Yao J, Paton AW 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

Yotnda P, Firat H, Garcia-Pons F, Garcia Z, Gourru G, Vernant JP, Lemonnier FA, Leblond V, Langlade-Demoyen P (1998a) Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. J Clin Investig 101:2290–2296

Yotnda P, Garcia F, Peuchmaur M, Grandchamp B, Duval M, Lemonnier F, Vilmer E, Langlade-Demoyen P (1998b) Cytotoxic T cell response against the chimeric ETV6-AML1 protein in childhood acute lymphoblastic leukemia. J Clin Investig 102:455–462

Zanetti M (2013) Cell-extrinsic effects of the tumor unfolded protein response on myeloid cells and T cells. Ann N Y Acad Sci 1284:6–11

Zhuang L, Scolyer RA, Lee CS, McCarthy SW, Cooper WA, Zhang XD, Thompson JF, Hersey P (2009) Expression of glucose-regulated stress protein GRP78 is related to progression of melanoma. Histopathology 54:462–470

Zou W (2006) Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol 6:295–307