Cell-extrinsic effects of the tumor unfolded protein response on myeloid cells and T cells

Maurizio Zanetti
The Laboratory of Immunology, Department of Medicine and Moores Cancer Center, University of California, San Diego, California

Address for correspondence: Maurizio Zanetti, The Laboratory of Immunology, Department of Medicine and Moores Cancer Center, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0815. mzanetti@ucsd.edu

Tumor-infiltrating myeloid cells, macrophages, and dendritic cells (DCs) are key regulators of tumor immunity and growth. The origin of tumor-derived signals that instruct myeloid cells in the tumor microenvironment is only partially understood. The endoplasmic reticulum (ER) stress response, or unfolded protein response (UPR), provides survival advantages to tumor growth. However, the cell-extrinsic effects of the tumor UPR on immune cells have not been explored. Our laboratory recently showed that the tumor UPR can be transmitted by yet unidentified factor(s) to myeloid cells, macrophages, and DCs. ER stress transmission to receiver myeloid cells upregulates the production of proinflammatory cytokines, and contextually of arginase I, leading to a proinflammatory/suppressive phenotype. DCs imprinted by tumor-borne ER stress transmissible factor(s) have decreased cross-presentation of antigen and defective cross-priming, causing T cell activation without proliferation. When DCs imprinted by transmissible ER stress are admixed with tumor cells and injected in vivo, facilitation of tumor growth is observed. Thus, tumor-borne ER stress plays a hitherto unappreciated role at the tumor/immune interface that ultimately facilitates tumor growth.

Keywords: ER stress; unfolded protein response; myeloid cells; dendritic cells; cross-priming; tumor growth facilitation; proinflammatory cytokines; arginase

Introduction

Eukaryotic cells react to endoplasmic reticulum (ER) stress by engaging a conserved set of intracellular signaling pathways known collectively as the unfolded protein response (UPR). ER stress response/UPR signaling pathways are activated in primary solid tumors as a result of cell-intrinsic defects, such as dysregulation of protein synthesis, folding and secretion, and aberrant glycosylation, but also microenvironmental noxae such as nutrient (e.g., glucose) deprivation, imbalance between demand and supply of oxygen (hypoxia), and imbalance between the production of reactive oxygen and the cell’s ability to readily detoxify reactive intermediates (oxidative stress). Some viruses that cause chronic infection also induce ER stress.

In mammalian cells, the UPR is initiated by three ER membrane-bound sensors, IRE1α, ATF6, and PERK, which, in unstressed cells, are maintained in an inactive state through luminal association with the ER chaperone molecule GRP78. When a cell experiences ER stress, GRP78 disassociates from each of the three sensor molecules to preferentially bind un/misfolded proteins, allowing each sensor to activate downstream signaling cascades, which act to normalize protein folding and secretion. PERK phosphorylates eIF2α, which results in selective inhibition of translation, effectively reducing ER client protein load. IRE1α autophosphorylates, activating its endonuclease to cleave Xbp-1 to generate a shortened Xbp-1 isoform (Xbp-1s), which drives the production of various ER chaperones to restore ER homeostasis. ATF6 translocates to the Golgi where it is cleaved into its functional form, which acts in parallel with Xbp-1s to restore ER homeostasis. If ER stress persists, downstream signaling from PERK via ATF4 can also activate the transcription factor,
Table 1. Links between the UPR and mechanisms of tumorigenesis

| Cell-intrinsic events                  | Cell-extrinsic events                  |
|----------------------------------------|----------------------------------------|
| Adaptation of cellular energetics      | Tumor promoting inflammation           |
| Genomic instability and mutation       | Evasion of antitumor immunity           |
| Insensitivity to antigrowth signals    | Promotion of angiogenesis               |
| Self-sufficiency in growth signals     |                                        |
| Replicative immortality (telomerase activation) |                           |
| Evasion of apoptosis                    |                                        |
| Tissue invasion and metastasis         |                                        |

C/EBP homologous protein (CHOP), which can initiate apoptosis. Thus, if ER stress is too intense or protracted, compensatory mechanisms fail and cells undergo apoptosis.3

Substantial evidence implicates the UPR in tumorigenesis and cancer progression.5 Breast cancers, possibly as a consequence of hypoxia, possess high levels of GRP78;6 and proliferating and dormant cancer cells in which GRP78 is upregulated are resistant to chemotherapy.7 The conditional homozygous knockout of Grp78 in the prostate of mice with Pten inactivation protects against cancer growth,8 whereas the inactivation, or a dominant-negative form, of PERK in cancer cells yields smaller and less aggressive tumors in mice.9 Moreover, the inactivation of PERK and IRE1α results in impaired tumor cell survival under hypoxic conditions in vitro and decreased tumor growth in vivo.9,10

The links between the UPR and tumorigenesis, cancer growth, and progression can be divided into two major categories. In the first category are the cell-intrinsic effects that include the more canonical links between the UPR and cancer, which are recognized to promote direct survival advantages (Table 1). The second category relates to the emerging role of the UPR in orchestrating inflammation in the tumor microenvironment, promoting evasion of antitumor immunity, and sustaining angiogenesis (Table 1). These cell-extrinsic effects are less clearly understood.

Although evidence that the UPR may regulate immunity is still limited,11 effects of the UPR on antigen presenting cells12 and T cells13,14 have been reported. Since both innate and adaptive immune responses play a crucial role in antitumor defense, and their subversion leads to tumor escape, it is important to define the role of the UPR in the context of antitumor immunity. This review will briefly recapitulate work from our laboratory, specifically highlighting the relation between the tumor UPR and immune cells, with emphasis on myeloid (CD11b+) and CD8 T cells. These studies initially focused on myeloid cells, owing to the fact that these cells, including macrophages and dendritic cells (DCs), infiltrate solid tumors, and under the influence of tumor-derived signals, polarize to a phenotype that facilitates tumor growth.15 In this context, tumor-infiltrating DCs inhibit T cell proliferation even though the signals necessary for efficient T cell priming are apparently in place.16,17

The work discussed here suggests that tumor-borne UPR is a new determinant of the tumor/immune interface, serving as a new interpretative tool for the phenomenology of the tumor microenvironment as it relates to tumor growth and progression.

A cell-extrinsic effect of tumor UPR on myeloid cells

A previously unappreciated cell-extrinsic effect of the tumor UPR is its transmission to myeloid cells, such as macrophages and DCs.18,19 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, lung carcinoma) on bone marrow–derived macrophages and DCs. Cancer cells were stressed using thapsigargin, a sesquiterpene lactone canonical ER stress inducer that inhibits the sarco/endoplasmic reticulum Ca2+ ATPase, and glucose starvation. We found that bone marrow–derived macrophages and DCs both function as receivers of TERS to which they respond by mounting a global ER stress response, characterized by transcriptional upregulation of the master UPR regulator Grp78 and two downstream UPR effectors, Xbp-1s and CHOP. Cancer cells under ER stress also upregulate a proinflammatory gene program, including the protumorigenic cytokines...
IL-6, IL-23p19, and TNF-α. Other cytokines/chemokines, such as TGF-β, MIP-1α, MIP-1β and MCP-1, are also increased, while there is no effect on IL-10. Importantly, TERS also caused the upregulation of arginase I in both macrophages and DCs. Since arginase I is a suppressor of T cell function, we concluded that TERS imparts myeloid cells with a proinflammatory/suppressive phenotype.

TERS-imprinted, bone marrow–derived DCs rapidly change morphology, acquiring characteristics of activated, mature DCs, including increased cell size and elongated dendrites. They also upregulate expression of major histocompatibility complex (MHC) classes I and II, and the costimulatory molecules CD86, CD80, and, to a lesser extent, CD40. These cells are CD8α−, confirming their myeloid origin. The general phenotypic features of CD11b+ cells, macrophages, and DCs upon TERS imprinting are summarized in Figure 1. TERS-imprinted myeloid cells do not upregulate GR-1, distinguishing their phenotype from that of classical myeloid-derived suppressor cells (MDSC).

Initial studies on fate determination of CD8 T cells cross-primed by TERS-imprinted DCs

Using the in vitro system described previously, we determined that T cells cross-primed by TERS-imprinted, bone marrow–derived DCs upregulate transcription of various cytokines, including IL-10 and TNF-α, but not IL-17. They also upregulate Foxp3 while downregulating CD28. LAG3, a negative regulator of TCR signaling on tumor-infiltrating CD8+ T cells, was only slightly upregulated. A provisional conclusion is that CD8 T cells cross-primed by TERS-imprinted, bone marrow–derived DCs display an uncommitted phenotype with potential suppressive characteristics detected by the monoclonal antibody 25-D1.16. Reproducibly, we found reduced display of the SIINFEKL/H2-Kb complex at the cell surface of OVA-fed, TERS-imprinted, bone marrow–derived DCs, while the expression of MHC class I molecules remained constant or even increased over that of OVA-fed control DCs. Using the OVA system and CD8+ T cells from OT-I mice whose T cell receptor (TCR) is specific for the SIINFEKL/H2-Kb complex, we investigated the ability of TERS-imprinting to affect CD8+ T cell cross-priming by bone marrow–derived DCs in vitro (Fig. 2, upper panel). As expected, OVA-fed, bone marrow–derived DCs, unstimulated or treated with the conditioned medium of unstimulated tumor cells, efficiently induced both activation and proliferation of OT-I T cells, as determined by surface staining for CD69, CD25, CD62L and CD44, and by 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) dilution. In contrast, OT-I T cells cocultured with OVA-fed, TERS-imprinted, bone marrow–derived DCs proliferated poorly while activated, resulting in a higher (>70%) percentage of activated, nondividing T cells. PD-1 was not upregulated in T cells. The proliferation defect could be restored by adding exogenous antigen (1 mg/mL), but not by adding exogenous IL-2 during cross-priming, arguing against classical T cell anergy. The proliferation defect was further rescued (~80%) by l-norvaline, a competitive inhibitor of arginase I, but surprisingly, not by the addition of l-arginine. Thus, tumor-borne UPR is transmitted to myeloid cells, which, in turn, produce arginase I, contributing to the T cell proliferation defect observed (Fig. 2, lower panel).
Characteristics of CD8\(^+\) T cells cross-primed \textit{in vitro} by TERS-imprinted, bone marrow–derived DCs (BMDCs). Upper panel: diagram illustrating the experimental procedure for cross-priming of OT-I CD8\(^+\) T cells using TERS-imprinted DCs. Lower panel: phenotypic and proliferative characteristics of CD8\(^+\) T cells cross-primed \textit{in vitro} by TERS-imprinted DCs. Control DCs comprise both DCs that received no treatment (regular cross-priming) and DCs treated with vehicle-conditioned medium. (–) = no appreciable variation from controls. Data shown with permission from Mahadevan \textit{et al.}\(^{18}\)

|                      | Control DC | TERS DC |
|----------------------|------------|---------|
| CD69                 | +++        | +++     |
| CD44                 | +++        | +++     |
| CD25                 | +++        | +++     |
| CD62L                | ±          | +       |
| CFDA-SE dilution     | +++        | +       |
| PD-1                 | –          | –       |
| LAG3                 | –          | –       |

(CD28 downregulation and IL-10 upregulation), which render them similar to CD8\(^+\)/CD28\(^-\) regulatory T cells secreting IL-10 and TNF-\(\alpha\), and expressing FOXP3 that have been found to infiltrate a variety of human tumors.\(^{27,28}\)

**Tumor growth facilitation**

In a direct test of whether immune cells facilitate tumor growth \textit{in vivo},\(^{29}\) we subcutaneously inoculated C57/BL6 mice with B16.F10 tumor cells admixed with TERS-imprinted, bone marrow–derived DCs; not surprisingly, this resulted in faster tumor growth, earlier tumor initiation, and decreased survival of mice.\(^{18}\) Furthermore, the inoculation of mice with TCI.OVA prostate cancer cells, constitutively expressing the OVA rejection antigen, caused transient tumor growth only when admixed with TERS-imprinted, bone marrow–derived DCs.\(^{18}\) This suggests that one of the effects of TERS is to induce dysfunctional CD8\(^+\) T cells, which ultimately may provide for immune escape by the tumor cells.
Conclusions

Recent work from our laboratory points to a role for the tumor UPR on myeloid cells and CD8+ T cells. The ensemble of these effects stems from the cell-extrinsic effects of the tumor UPR, and represents a new way through which tumor cells, under the umbrella of the UPR, can affect both myeloid cells and CD8+ T cells. Paradoxically, the defect of bone marrow–derived DCs deriving from TERS imprinting differ from that of tolerogenic DCs, which have been described as steady-state immature cells able to present antigen. In contrast, TERS-imprinted, bone marrow–derived DCs are phenotypically mature cells with diminished capacity of cross-presenting antigen and cross-priming CD8+ T cells. Thus, cell-extrinsic signals borne out of tumor UPR can recapitulate ab initio the activated/suppressive phenotype observed in tumor-infiltrating myeloid cells in vivo. The extent to which TERS-mediated functional incapacitation of myeloid cells induces immune evasion is not known at this time and will require further work. However, because the tumor UPR induces, through TERS, several of the immune defects observed in the tumor microenvironment, new attention must be directed at therapies that target the UPR to effectively curb tumor cells adaptation and survival in vivo and to control immune suppression due to the cell extrinsic effects of the tumor UPR (Fig. 3). It is not difficult to imagine that this new type of control of the tumor microenvironment will effectively aid the generation and persistence of spontaneous, or vaccine-induced, antitumor T cell responses.

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Conflicts of interest

The author declares no conflicts of interest.

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Figure 3. Diagram illustrating the potential benefits of therapeutic interventions directed at the tumor UPR and its cell-extrinsic effects such as TERS.
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