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Addicted to secrete – novel concepts and targets in cancer therapy

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The unfolded protein response (UPR) mediates the adaptation of the secretory pathway (SP) to fluctuations in cellular protein demand or to environmental variations. Recently, drug screenings have confirmed the therapeutic potential of targeting the UPR in cancer models. However, the UPR may not be the only druggable target of the SP. Moreover, recent studies have revealed other contributions of the SP to cancer development. This article does not intend to describe the well-established implication of UPR signaling pathways in cancer cell life and cell decision, but rather aims at defining the concept of 'tumor cell secretory addiction', from molecular, cellular, and therapeutic perspectives. Furthermore, the implication of UPR modulations in this context will be discussed.

Overview of the secretory pathway in cancer

Approximately one-third of the polypeptides synthesized by a cell enter the endoplasmic reticulum (ER), the first compartment of the secretory pathway (SP) (see Glossary). From there, the polypeptides undergo different maturation steps including folding, glycosylation, and disulfide bond formation, thereby enabling the properly folded proteins to exit the ER and reach their final destination. These multistep and active processes are tightly regulated and controlled to maintain ER protein homeostasis (or ER proteostasis) [1,2]. Any physical, chemical, or biological conditions that disturb this efficient ER proteostasis network are rapidly sensed as part of the cell stress response. The main signaling pathway governing these molecular mechanisms is collectively known as the

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Keywords: unfolded protein response; secretory pathway; cancer therapy.

1471-4914/$ – see front matter
 http://dx.doi.org/10.1016/j.molmed.2013.12.003

Glossary

Aneuploidy: condition in which a cell has an incorrect number of chromosomes.
Autophagy: an intracellular degradative process that delivers cytoplasmic materials to the lysosome for degradation.
Endoplasmic reticulum (ER): the first component of the cell SP; it encompasses an extensive network of membrane tubules, vesicles, and flattened cisternae within the cytoplasm. The main function of the ER is to serve as a platform for lipids, or carbohydrate metabolism, secretory protein folding, and maturation and control of cell calcium homeostasis.
Glycosylation: post-translational modification process in which carbohydrates (glycans, saccharides, or sugars) are covalently attached to proteins, lipids, or other organic molecules.
Golgi apparatus: membranous organelle and component of the cell SP that receives the output (proteins and lipids) from the ER and allocates them to their final destinations. It also acts as a reaction chamber for specific protein glycosylations, phosphorylations, and cleavages.
Immunogenic cell death: a cell death subroutine that, in contrast to the physiological caspase-mediated-tolerogenic apoptosis, stimulates cancer cell immunogenicity along with a protective anticancer immune response *in vivo*.
Oncogenesis: all the events which will lead to the transformation of normal cells to cancerous cells.
Proteostasis: refers to a proper balance among synthesis, maturation, and degradation of cellular proteins.
Proteotoxic compounds: compounds that alter proteostasis.
Secretome: the collection of all molecules secreted by a cell, including trophic factors and immunomodulatory cytokines.
Secretory pathway (SP): the complex network of eukaryotic cell organelles that mediates the folding, maturation, and trafficking of transmembrane and secreted proteins. It is also responsible for biogenesis and proper intracellular distribution of a wide range of complex carbohydrates and lipids.
Tumor cell secretory addiction: describes the need for cancer cells to possess a particularly efficient SP. This need can be the result of (i) an increase in secretory protein demand (due to aneuploidy or oncogene expression); (ii) a metabolic crisis associated with cancer cell high metabolism and energy depletion; or (iii) high levels of proteotoxic stresses mediated by basal oxidative stress or chemotherapy. Furthermore, this need creates a dependency of cancer cells for the SP and makes the SP an interesting therapeutic target.
Unfolded protein response: an evolutionarily conserved cellular response that mediates the adaptation of the SP to fluctuations in the cellular protein demand or to environmental variations. It encompasses a set of distinct signaling pathways that aim at reducing global protein load to the ER and increasing the levels of chaperones and the degradation of misfolded proteins.

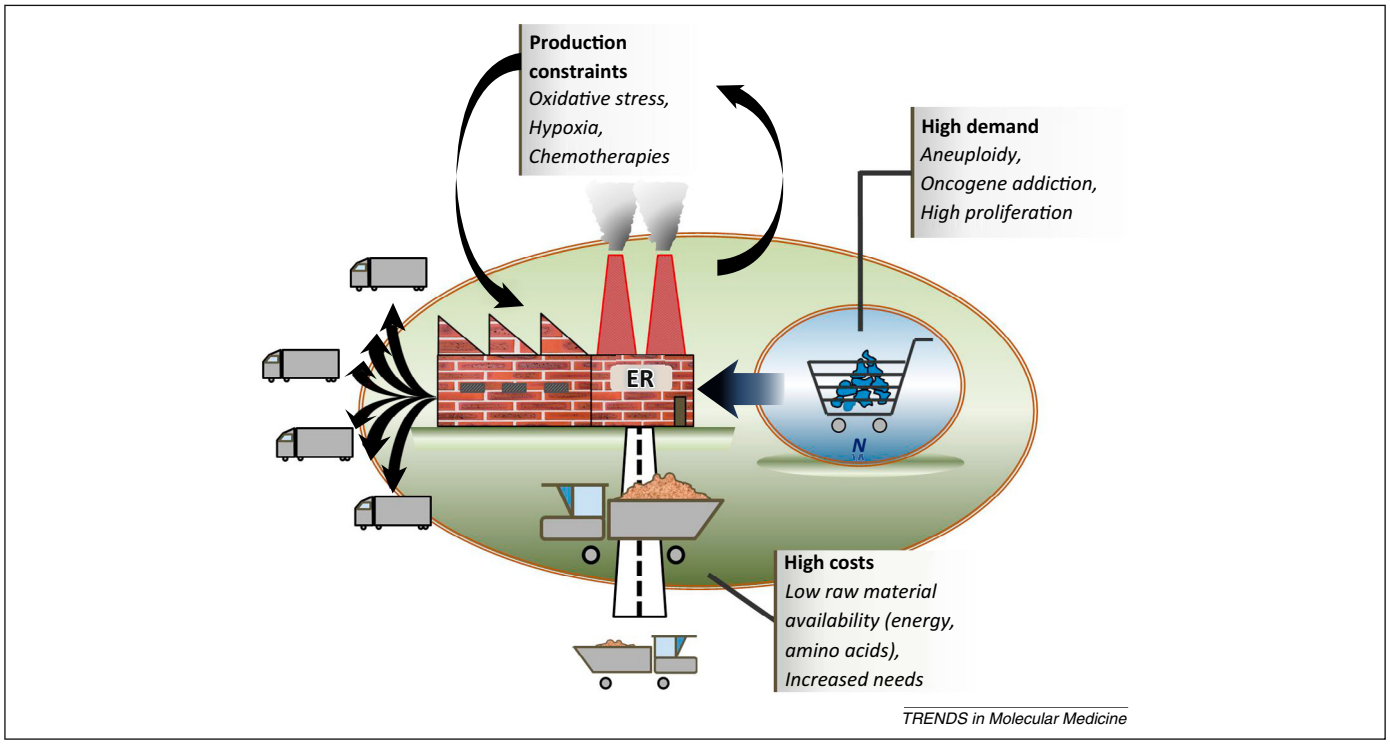

Figure 1. Secretory protein demand, cost, and price in cancer: the secretory crisis. The secretory pathway can be considered as a whole as one of the 'factories' of the cell. In this view, production of secreted proteins is dependent on the demand, dictated by the nucleus, the availability of raw materials (e.g., energy, amino acids), and production constraints. Critical factors destabilizing this system in cancer cells are indicated. Abbreviations: N, nucleus; ER, endoplasmic reticulum.

unfolded protein response (UPR) and is initiated by three ER transmembrane proteins in mammals, namely, activating transcription factor 6 (ATF6), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), and inositol-requiring enzyme 1 (IRE1) [3]. Thus, the UPR aims at restoring ER homeostasis under stressful conditions, and if this fails then cell death is engaged.

By nature, features of cancer cells, such as aneuploidy, increase in metabolic demand, and high proliferation rates, require particularly robust ER and secretory machineries. This increased demand for the secretory functions in tumors is likely to trigger an alteration of ER homeostasis and consequently result in 'basal' or constitutive UPR induction (Figure 1). Moreover, in certain cancer types, SP-associated functions have been identified as fundamental players in oncogenesis. As such, the UPR plays prooncogenic or antioncogenic roles depending on the tumor context. It can be tumor suppressive in early stages of cancer development, as reported in melanoma or Ras-driven lung cancer [4,5], whereas it can be tumor promoting in established cancer, such as in multiple myeloma (MM) [6] and other cancers [7,8]. Moreover, beyond the general increase in the demand that challenges the SP, the secretion of prooncogenic factors, such as growth factors and their associated receptors, might also confer specific dependency of the cancer cells towards the SP. This is well illustrated by the interaction of tumor cells with their environment [9]. Indeed, tumor–stroma interactions are controlled by cytokines, extracellular matrix (ECM) proteins, matrix metalloproteases (MMPs), integrins, and other contact proteins, all trafficked to their final destination through the SP. Modulation of the SP by endogenous or exogenous stress factors could therefore result in cell ECM destabilization and an increase in cancer dissemination and invasion. Finally, the relation between the SP and oncogenesis is illustrated by the increasing number of reports describing a role for plasma membrane exposure of major ER-resident proteins in tumor immunogenicity [10,11]. All these factors have prompted us to reconsider the contribution of the tumor cell secretory machinery in cancer development and progression.

The economics of secretory protein production: cost, demand, and price to pay in cancer cells

Aneuploidy is a hallmark of cancer cells that contributes to tumorigenesis by providing gain of oncogenes or loss of tumor suppressor genes [12]. Adding to that, an increase in the number of chromosomes was found to induce hypersensitivity to conditions interfering with protein synthesis and protein folding in yeast [13], and in human cancer cells [14]. This suggests that aneuploidy-mediated translation increase is an Achilles' heel of cancer cells because it alters proteostasis. Along these lines, an increase in ploidy was recently found to be associated with ER stress in cancer [7]. Consequently, interfering with proteostasis represents an appealing strategy to target cancer cells specifically (Figure 2). Moreover, the aneuploidy-induced SP challenge is also associated with immunogenicity, due to aberrant cell surface exposure of calreticulin (CRT).

However, the dependence of cancer cells to the SP is not restricted to aneuploidy and a variety of cancer-associated molecular processes are responsive to alterations in the secretory machinery. These include, but are not limited to, high proliferation, elevated metabolism, and oncogene-mediated increases in protein demands. Indeed, Huber *et al.* found that cell transformation results in an increase

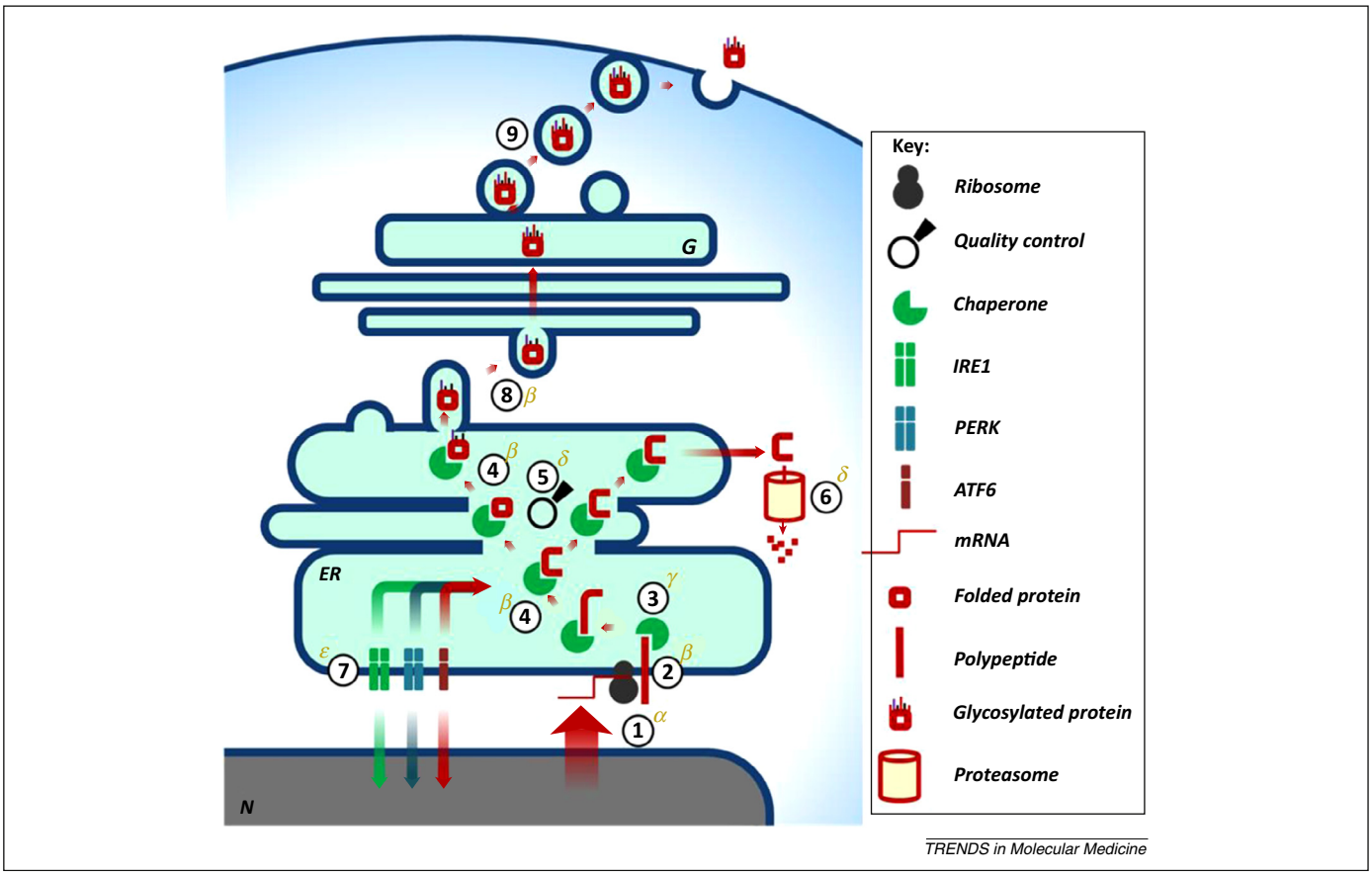

Figure 2. Targeting the secretory pathway in cancer. Proteins that are destined for secretion are translocated into the ER lumen co-translationally (1) through channels called translocons (2). In the ER lumen, the polypeptides are then matured through a complex process involving resident chaperones (3). Protein maturation includes the proper folding of the nascent protein, N-linked glycosylation, proline isomerization, and disulfide bond formation (4). The ER quality control system will ensure the correct folding of proteins (5) and will orient misfolded proteins in a 'repair' or 'refolding' process or will target these proteins for retrotranslocation to the cytosol and degradation by the proteasome (6). Every condition that destabilizes this fine equilibrium in favor of the accumulation of misfolded proteins in the ER will trigger the activation of the three ER stress sensors, IRE1, PERK, and ATF6, thus initiating the unfolded protein response (7). Once correctly matured in the ER, the nascent proteins will exit the ER and be transported in a coatomer protein complex II (COPII)-dependent manner to the Golgi complex (8). After another set of maturation in this organelle, proteins will continue to move on through the secretory pathway and will be secreted or transported to their final destination (9). Therapeutic intervention might therefore be possible at many levels in the secretory pathway in cancer cells, including: (α) decreasing the amount of proteins entering the secretory pathway and, as a consequence, counteracting the increased demand of secretory proteins mediated by hyperploidy or oncogene activation; (β) modulating the secretion of select proteins, such as receptor tyrosine kinases, growth factors, or cytokines, by targeting their traffic through the secretory pathway, or protein specific processing, such as N-linked glycosylation; (γ) targeting ER chaperones, such as BiP or GRP94, for decreasing chemoresistance, or the levels of their respective client proteins; (δ) increasing proteostasis unbalance, for example, by inhibiting ERAD, proteasomal degradation, or modulating protein quality control; and (ε) modulating the adaptive response of the secretory pathway, the UPR, to sensitize cancer cells to ER stress and cancer treatment. Abbreviations: N, nucleus; ER, endoplasmic reticulum; G, Golgi; ATF6, activating transcription factor 6; PERK, protein kinase R (PKR)-like endoplasmic reticulum kinase; IRE1, inositol-requiring enzyme 1; BiP, immunoglobulin heavy chain-binding protein; GRP94, 94 kDa glucose-regulated protein; ERAD, endoplasmic reticulum-associated protein degradation.

in proliferation and metabolic demand, thereby leading to glucose depletion and consecutive ER stress [5]. Furthermore, Myc-mediated protein synthesis is necessary for the increase in cell size and for acceleration of cell cycle progression of a transformed cell [15]. By exploiting this concept of cancer dependency towards the SP, De Raedt *et al.* showed that targeting proteostasis is a good strategy for treating Ras-driven cancers [8]. Considering the relation between cancer cell features and the SP, it is not surprising that a plethora of publications report overactivation of UPR signaling in most human tumors [16]. Furthermore, apart from the intrinsic demand of the cell, the UPR can also be induced by environmental challenges in tumors (Figure 1). These stresses were widely described over the past few years and include hypoxia, oxidative stress, and chemotherapies [16,17]. The integration of both intrinsic and extrinsic challenges therefore results in a selective pressure to which cancer cells dynamically adapt to maintain their selective advantage. As such, the well-described upregulation of ER chaperones in tumors could be seen as a consequence of this integrated/global stress where the cancer phenotype is driven by a spontaneous adaptation to the fluctuating environment.

The efficiency and plasticity of the SP represents a critical factor in prooncogenic processes. Indeed, several oncogenic proteins depend on the SP for proper folding and to reach their final destination. This is the case of growth factors or cancer-associated receptor tyrosine kinases such as v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2) [18,19]. Moreover, oncogenes such as v-myc avian myelocytomatosis viral oncogene homolog (Myc) and Kirsten rat sarcoma viral oncogene homolog (K-RAS) require an increase in the protein flow through the SP to mediate their oncogenic potential [8,20]. The best example of this is illustrated by the role of Myc as a universal amplifier of transcription [21,22], combined

with the general increase in protein synthesis rates necessary for acceleration of Myc-induced cell cycle progression and for Myc oncogenic potential [15]. This results in increased protein supply to the ER, leading to ER stress and UPR activation in lymphomas [20]. In this model, UPR activation and, particularly, the PERK/eukaryotic translation initiation factor 2α (eIF2α)/activating transcription factor 4 (ATF4) axis increase autophagy and protect cancer cells against ER stress-dependent apoptosis. Moreover, Myc neuroblastoma derived homolog (MYCN) activation in neuroblastoma creates a dependency towards glutamine metabolism, and glutamine deprivation induces cell death through eIF2α/ATF4 signaling [23]. In this model, eIF2α/ATF4 activation was triggered by the eukaryotic translation initiation factor 2 alpha kinase 4 (GCN2) independently of PERK. Hence, depending on the cancer or on the stress, activation of ATF4 appears as a central player in the control of Myc-mediated oncogenesis.

Interactions of cancer cells with their environment

Most of the ECM and contact proteins are matured and trafficked through the SP. As a consequence, perturbing the SP could result in the alteration of the microenvironment of tumor cells. The role of the ECM in cancer development has led to the observation that tissue architecture can control gene expression and determine the malignancy of cancer *in vivo* [9]. Thus, overcoming the tumor suppressor functions of the environment requires 'promotion' by other elements. In this hypothesis, ECM destabilization by modulation of the SP could represent one of these important promoting factors. Although still to be validated, this suggests that mutations or deregulation of major components of the SP could act as cancer drivers. Several pieces of evidence in support of such a phenomenon have emerged in recent years. An example is provided by the *IRE1* gene, coding for one of the three mediators of the UPR. Somatic mutations in this gene have been identified in various cancers [24–26]. In a study by Greenman *et al.*, *IRE1* was ranked as the fifth highest mutated kinase, carrying at least one driver mutation [24]. Furthermore, expression of a dominant negative form of IRE1 triggered a mesenchymal drift in glioblastoma [27,28] and altered the expression of ECM, angiogenesis, and inflammation proteins [27,29,30]. As a consequence, cancer invasion or angiogenesis appear dependent on a functional SP in tumor cells. Thus, alteration of the expression or function (mutation) of an important gene that regulates secretory homeostasis should be sufficient to modulate tumor features such as growth, invasion, or angiogenesis.

Adding to modulation of IRE1 activity, the expression levels of Sec23 homolog A and homolog B (SEC23A and B) proteins, members of the coatomer protein complex II (COPII) machinery, were found to be instrumental in changing the tumor cell secretome in various cancers [31,32]. The main effect was to modulate the tumor microenvironment and promote tumor cell metastasis. Moreover, the signal peptidase complex 18 (SEC11A) contributes to progression of gastric cancer via transforming growth factor-α (TGF-α) secretion [33].

In addition, cancer cells actively secrete immunosuppressive or protumorigenic cytokines. In this context, the tumor UPR, stimulated by the hostile cancer microenvironment, is a plastic modifier of the tumor immune and inflammatory landscape [34,35]. An interesting paradigm delineating how the cancer cell itself may assist in the maintenance of a proinflammatory and protumorigenic tumor environment through a non-cell autonomous mechanism modulated by 'transmissible' ER stress has also recently been described [34]. In this model, murine tumor cells stressed by thapsigargin [a sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pump inhibitor] were capable of transmitting signals to co-cultured macrophages, which, in turn, underwent ER stress and UPR activation. This was associated with the production of proinflammatory and protumorigenic cytokines by macrophages, including interleukin 6 (IL-6), IL-23, tumor necrosis factor-α (TNF-α), and the upregulation of arginase, which have been implicated in jeopardizing antitumor immunity responses [34]. Thus, tumor addiction to the SP appears to exploit the ER stress response beyond cell autonomous frontiers. Although the precise mechanism(s) underlying non-cell autonomous ER stress remains unclear (Box 1), it is interesting to note that the ER luminal domain of an ER stress transducer, the cAMP responsive element binding protein 3-like 2 (CREB3L2), that shares sequence similarity with ATF6, is secreted into the extracellular space as a signaling molecule and plays a role in cell-to-cell communication [36]. Additionally, two other ATF6-like ER stress transducers, old astrocyte specifically induced substance (OASIS) and cAMP responsive element binding protein 3-like 4 (CREB4), were also found to be secreted. Understanding the functions of these secreted ER luminal domains might unravel mechanisms of UPR non-cell autonomous communication. Finally, it is interesting to highlight the fact that non-cell autonomous control of the UPR has been demonstrated in other models *in vivo* [37,38]

Post-translational modifications in the secretory pathway of the cancer cell

The SP and, in particular, the Golgi apparatus are also important for the control of glycosylation. It has long been reported that tumor cells frequently display marked alterations in their glycosylation profiles. For example, more than 80% of epithelial-derived malignant tumors express the Tn antigen [39] and a similar proportion express the T antigen [40]. Tn and T are related O-glycans. Tn is composed of one sugar residue, GalNac, linked to a Ser or Thr residue, and T corresponds to Tn with an additional galactose residue. Their levels of expression are strongly correlated with tumor aggressiveness and poor clinical outcome [41]. Recently, we have shown that high Tn expression in tumors is due to a reorganization of the SP. In malignant cancer cells, the GalNac-transferases (GALNTs), responsible for the initiation of O-glycosylation are redistributed from the Golgi apparatus to the ER [42]. This relocation results in large amounts of Tn antigen in the ER. The GALNT relocation appears tightly regulated and is induced by the activation of the Src tyrosine kinase [43]. Remarkably, between 60% and 80% of human breast tumors show a large degree of relocation of GALNTs to the

ER, whereas in normal tissues the enzymes are generally exclusively localized in the Golgi apparatus [44]. The relocation induces a marked stimulation of migration and tissue invasion, which probably explains why Tn levels are strongly correlated with tumor aggressiveness [44].

O-Glycosylation initiation in the ER results in modifications of cell surface glycoproteins. However, it is not clear at this time what these proteins are nor how they might mediate tissue invasion. Recent studies have revealed that hundreds of different proteins are O-glycosylated and some ER-resident proteins have been shown to bear this modification [45]. Multiple specific examples of the regulation of secreted and cell surface factors by O-glycans have also started to emerge. A recurrent theme is that O-GalNac glycans can regulate protein hormones and other secreted factors by inhibiting proteolytic cleavage [46]. For example, FGF23 is a peptidic hormone that controls systemic phosphate metabolism [47] and O-glycosylation of FGF23 is required for secretion and protection against inhibitory cleavage by proprotein convertases [46]. Numerous other secreted factors have been shown to undergo similar regulation [46].

Another recent study has also highlighted the extent of regulation of glycan synthesis by various signaling pathways at the level of the Golgi apparatus [48]. These results suggest that various aspects of cell physiology could be regulated by select signaling intermediates through the SP and the synthesis of glycoproteins. As these are often perturbed in cancer, it is possible that many of the glycophenotypes of cancer arise from a perturbation at the interface between signaling and the SP.

Another important protein post-translational modification in the SP is the formation of disulfide bonds. Considering that the redox balance is generally affected in cancer cells [49], therefore, the control of ER redox state could have considerable implications for this oxidative process and for protein folding and assembly in this pathology. In many instances, the expression of protein disulfide isomerases or oxidoreductases was found to be deregulated in cancers [17]. This is the case for endoplasmic reticulum protein 29 (ERp29), protein disulfide isomerase family A, member 6 (PDIA6), or protein disulfide isomerase family A, member 4 (PDIA4) [17]. In addition, the overexpression of quiescin sulfhydryl oxidase 1 (QSOX1), an enzyme that oxidizes thiols during protein folding, has been observed in cancers of breast, pancreas, and prostate origins [50–53]. QSOX1 can also be secreted and in the extracellular space it acts in conjunction with PDIs to modify the ECM [54]. As a consequence, deregulated expression of QSOX1 has also been proposed to be important during tumor cell invasion, facilitating tumor cell migration at the tumor/stroma interface [54]. As such, QSOX1 may be a prognostic indicator of metastatic potential or even indicate that cancer is present in a host. In this line of evidence, inhibitors of PDIs have potent antitumor activities [55,56]. Proteostasis disturbances have also been linked to excessive oxidative stress, which could therefore enhance genomic instability [57]. As such, it is believed that the existence of a vicious circle between altered protein folding and local reactive oxygen species (ROS) production at the ER enhances misfolding [58]. This may translate into prooncogenic cascades, in which ATF4 downstream signaling pathways control the redox balance of the cell [59,60].

Proteolytic processing in the SP also represents an interesting feature in cancer. The activation of membrane-anchored transcription factors such as ATF6, cAMP responsive element binding protein 3-like 3 (CREBH), OASIS, or sterol regulatory element binding transcription factors 1 or 2 (SREBP1/2) through their perimembrane cleavage in the Golgi complex by site 1 and site 2 proteases (S1P and S2P) [61] could be involved in the ability of the cancer cell to handle proteotoxic stress. Moreover, convertase-mediated processing of vascular endothelial growth factor C (VEGF-C) and platelet-derived growth factor alpha (PDGF-A) has been shown to confer these growth factor protumoral and proangiogenic properties [62–64]. Finally, another illustration of such activation is provided by the recent discovery that caspase-8-mediated cleavage of the calnexin cytosolic domain prompted the release of a fragment interfering with epidermal growth factor receptor (EGFR)/signal transducer and activator of transcription 3 (STAT3) signaling, a pathway canonically overactivated in various cancers [65].

Secretory addiction and cancer immunogenicity

To progress towards a full malignant phenotype, cancer cells need to evade the host immune system. Through a process widely known as tumor immunosurveillance, nascent immunogenic cancer cells are eliminated by various components of the innate and adaptive immune systems, before they can outgrow and form detectable tumors. Mechanisms empowering cancer cells to dampen their immunogenicity and gain immunoevasive abilities allow the clonal expansion of low immunogenic or 'immunoedited' cancer cells [66]. A recently described mechanism intrinsic to cancer cells favoring immune surveillance links polyploidization (one of the initiating events of carcinogenesis) [67] to constitutive ER stress (surface exposure of the ER luminal chaperone CRT) [7]. Conditions promoting hyperploidy in various cancer cell lines prompted ER stress and led to sustained PERK-associated eI2Fα phosphorylation, which then caused the mobilization of CRT to the cell surface [7]. Increased or aberrant surface expression of CRT, in cooperation with other immunomodulatory factors, acts primarily as a recognition signal, facilitating the phagocytosis of the stressed or dying cancer cells by antigen presenting cells (APCs), such as dendritic cells [11,68].

In fact, the ability of stressed or dying cancer cells to surface translocate and/or secrete a crucial set of immunogenic danger signals, such as CRT and ATP, has been found to be critical for mounting an effective antitumor immune response *in vivo*. This could be connected to the recent discovery of CRT mutations in myeloproliferative neoplasms with nonmutated JAK2 [69,70]. It is also important to note that, adding to the discovery of aberrant surface exposed CRT in cancer, other ER chaperones such as immunoglobulin heavy chain-binding protein (BiP), 94 kDa glucose-regulated protein (GRP94), endoplasmic reticulum resident protein 57 (ERp57), and PDI have been shown to be secreted or expressed at the surface of cancer cells, where they can also induce antitumor immune responses. This particular point [10] expands the role of

the ER chaperones in tumorigenesis. Future studies are needed to determine if these key ER chaperones cooperate to generate a set of danger signals to enhance immunogenicity and the molecular mechanisms involved in establishing these signals (Box 1).

'Immunogenic cell death' (ICD) is coupled to, and depends on, the spatiotemporal surface expression and secretion of danger signals, collectively known as damage-associated molecular patterns (DAMPs) by stressed or dying cancer cells [11]. ICD is predominantly governed by loss of ER proteostasis, induction of oxidative stress, and the trafficking and release/externalization of DAMPs requires ER-to-Golgi transport and the classical SP. Interestingly, loss of proteostasis and ER stress are also common responses to viral infection, one of the most ancient host–cell interactions capable of communicating danger, and certain oncolytic viruses instigate ICD through their ability to cause ER stress (reviewed in [35]). Notably, the PERK pathway is required to signal ICD [11,71,72]. Thus, it is tempting to speculate that the capability of a specific 'ER stressor' to induce a robust PERK-mediated 'viral response-like' gene expression program is the key to forcing efficient DAMPs in cancer cells, thereby communicating a state of 'danger' to the immune system of the host.

Implications for secretory pathway addiction in cancer therapy: looking for a cure or an overdose?

In non-tumor cells, the UPR is fundamental for driving and orchestrating adaptation of the secretory machinery to constant fluctuations in secretory demand. As discussed above, most cancer cells show an increased expression of proteins of the secretory machinery, including ER chaperones. The overexpression of these proteins could have multiple consequences for cancer cell chemosensitivity. First, it creates cancer cell dependence towards some proteins central to ER functions, which therefore constitute potential therapeutic targets for cancer treatment (Figure 2). Recently, this concept has led to the application of ER stress targeting drugs to preclinical models of cancer (reviewed in [73]). For instance, the IRE1 inhibitor STF083010 was tested on a model of human MM with

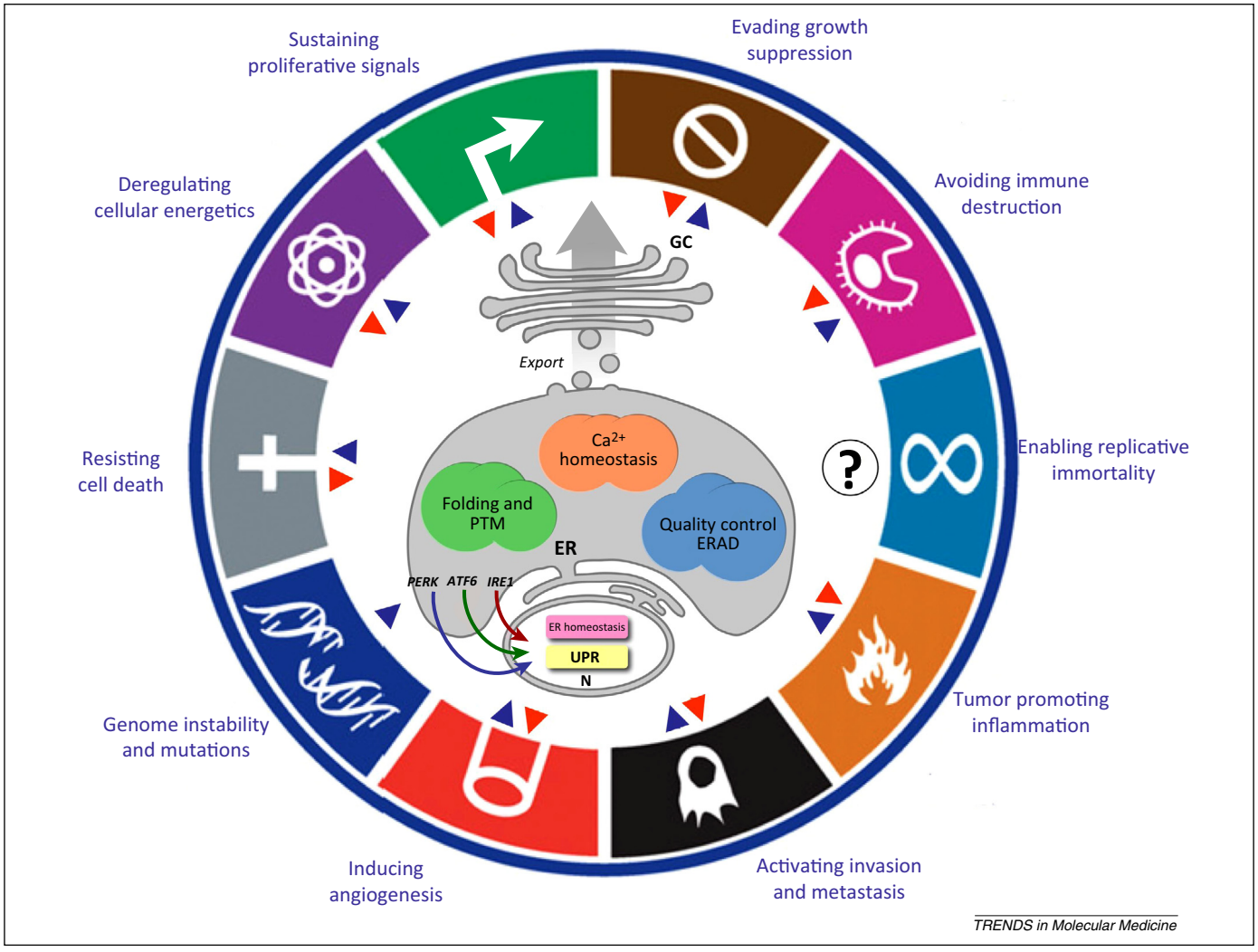

Figure 3. Impact of tumor cell secretory functions on the hallmarks of cancer. Schematic representation of the interactions of the secretory pathway (SP) with different hallmarks of cancer as defined by Hanahan and Weinberg [91]. The different molecular machines of the endoplasmic reticulum (ER) are indicated: folding and post-translational modification (PTM) in green, ER-associated degradation and quality control in blue, and calcium homeostasis in orange as well as the three arms of the unfolded protein response (UPR). The nucleus (N) and Golgi complex (GC) are also indicated. Arrows indicate either positive (blue) or negative (orange) regulatory mechanisms linking the SP to the hallmarks of cancer. The question mark indicates an absence of information available in the literature on the relation between the SP and replicative immortality.

great efficacy [73,74]. The PERK inhibitor GSK2606414 was also recently identified and displays potent antitumor and antiangiogenic activities in a xenograft model of pancreatic cancer [75]. Second, overexpression of the secretory machinery could also contribute to the ability of cancer cells to resist chemotherapies, because the simple overexpression of the central chaperone BiP has been shown to be protective against a wide range of chemotherapeutic agents [76].

The control of ER proteostasis could also represent a mechanism for forcing cancer cells to enter a secretory crisis [8,14]. This concept has been studied in MM, where cells are characterized by high protein synthesis resulting in chronic ER stress and IRE1 activation [77]. In this cancer, salubrinal, an inhibitor of eIF2α phosphatases [78], was reported to synergize with proteasome inhibitors (PIs) by specifically targeting surviving quiescent cells that present attenuated eIF2α phosphorylation [79]. Moreover, two IRE1 inhibitors, MKC3946 and toyocamycin, were also reported to synergize with PIs [73]. The molecular mechanism proposed is that both PIs and IRE1 inhibitors can increase basal ER stress in MM by different processes, the former by increasing accumulation of misfolded ER-processed proteins [80], the latter by blocking ER-adapting pathways. By contrast, the absence of X-box binding protein 1 (XBP1), a major UPR transcription factor activated by IRE1, was recently found to promote PI resistance in MM [81]. In this study, the authors found that the absence of XBP1 causes decommitment to Ig production and a decline in ER stress, thus leading to attenuation of MM ER stress hypersensitivity. Therefore, IRE1/XBP1 could be involved in both acute response and cancer adaptation and therapies combining IRE1 inhibitors and PIs should be used carefully. Finally, another potential therapeutic example is provided by the development of GRP94 inhibitors [82], which could be particularly applicable in recurrent breast tumors where increased GRP94 expression is not associated with PERK activation [83].

Large-scale studies have brought a significant amount of information on the nature and regulation of the secretory machinery [48,84–86]. One opportunity for novel cancer therapy could arise from proteins specialized in the secretion of specific proteins, or from the characterization of the specificity of ER chaperones. One example is trafficking protein particle complex 2 (Sedlin), which functions in the specific export of procollagen from the ER [87]. A therapeutic strategy targeting Sedlin could be applied to cancers in which collagen plays an important role, for example, in Ras-driven human epidermal tumorigenesis where collagen VII has been shown to be required for tumor cell invasion, or in fibrotic breast tumors where collagen crosslinks and consecutive ECM stiffness is associated with malignancy [88,89]. Similarly, fine-tuning cotranslational translocation to the ER can affect cancer-related secreted and transmembrane proteins in a selective manner [18]. Finally, better understanding of the receptor tyrosine kinase (RTK) secretory maturation will provide new therapeutic opportunities, particularly relevant in tumors presenting multiple RTK amplifications [90].

Box 1. Outstanding questions

- Do ER chaperones (such as CRT, BiP, GRP94, and PDI) cooperate to generate a set of danger signals to enhance immunogenicity?
- What are the mechanisms that link post-translational protein modifications in the SP to cancer development? Do they constitute a cause or a consequence of SP destabilization in tumors?
- What are the molecular mechanisms involved in 'transmissible/nonautonomous' ER stress?
- How can tumors be classified by the prooncogenic or antioncogenic role of their SP to adapt therapies targeting these processes?
- Is the general increase in levels of ER chaperones in tumors the result of stress states or adaptive processes? How can these two UPR-hypersensitive or UPR-resistant states be distinguished in order to orient therapies targeting proteostasis?
- Can therapies targeting specific processes of the SP be designed and developed to selectively target prooncogenic secreted and transmembrane proteins?

Concluding remarks

In conclusion, we are now only beginning to perceive the implications of the SP functions and adaptation in tumors, in tumor initiation, growth, and metastasis (Figure 3). Recent studies have shown that oncogenesis itself can modulate the whole SP, and that adaptation towards this pathway appeared as a key event in tumor evolution. Furthermore, UPR adaptation could make cancer cells both more adaptable and hypersensitive to specific stresses and chemotherapies. In certain cancers, ER stress constitutes a selective pressure barrier that cancer cells have to bypass to develop. Then, the SP could have opposite functions depending on the tumor evolution. Thus, it appears promising to integrate this complexity in the search of specific anticancer therapies that target the SP.

Acknowledgments

This work was supported by funding from the Institut National du Cancer (INCa), Institut National de la Santé et la Recherche Médicale (Inserm), Fondation de France, and La Ligue contre le Cancer_comité des Landes (E.C.); Millennium Institute no. P09-015-F, ACT1109, FONDEF D11I1007, FONDECYT no. 1110987 (C.H.), and Breast Cancer Campaign (2010NovPR13). N.D. was supported by a postdoctoral fellowship from the Fondation de France.

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