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Abstract

Epithelial cancer patients that are not considered eligible for surgical resection frequently benefit from chemotherapy. Chemotherapy is the treatment of cancer with one or combination of cytotoxic or cytostatic drugs. Recent advances in chemotherapy allowed a great number of cancer patients to receive treatment with significant results. Unfortunately, resistance to chemotherapeutic drug treatment is a major challenge for clinicians in the majority of epithelial cancers because it is responsible for the inefficiency of therapies.

Mucins belong to a heterogeneous group of large *O*-glycoproteins that can be either secreted or membrane-bound. Implications of mucins have been described in relation to cancer cell behavior and cell signaling pathways associated with epithelial tumorigenesis. Because of the frequent alteration of the pattern of mucin expression in cancers as well as their structural and functional characteristics, mucins are thought to also be involved in response to therapies. In this report, we review the roles of mucins in chemoresistance and the associated underlying molecular mechanisms (physical barrier, apoptosis resistance, drug catabolism or exclusion, cell stemness, epithelial mesenchymal transition) and discuss the therapeutic tools/strategies and/or prognosis biomarkers for personalized chemotherapy response that could be proposed from these studies.

Keywords: mucin, cancer, resistance, chemotherapeutic drug, apoptosis, prognosis biomarker

Abbreviations

PDAC: Pancreatic ductal adenocarcinoma

CRC: Colorectal cancer

EMT: Epithelial mesenchymal transition

MUC1-CT: MUC1 cytoplasmic tail

N-t : Amino-terminal

C-t : Carboxy-terminal

PTS: Proline Threonine Serine

1. Introduction

Mucins belong to an heterogeneous group of large *O*-glycoproteins composed of a long peptidic chain (called apomucin) on which are linked hundreds of oligosaccharidic chains. Initially, the mucin word designated glycoproteins secreted by specialized epithelial cells, the goblet cells, as part of the mucus gel. Mucins were biochemically characterized as massive molecules with high molecular weight able to form viscoelastic gels and responsible for the rheological properties of mucus [1]. The molecular era that led to genome sequencing allowed the classification of two sub-groups of mucins: (i) secreted mucins that mostly complied with this definition and (ii) membrane-bound or transmembrane mucins that did not fit in. Despite this dichotomy, mucins were all included in the MUC family with the approval of Human Genome Organization Gene Nomenclature Committee (HUGO/GNC) [2].

Secreted mucins are the major components of viscoelastic mucus gels and form a tridimensional network that protects the epithelia against various agression (inflammation, bacteria, virus, pollutants, pH, etc). This subgroup mainly includes: MUC2, MUC5AC, MUC5B, MUC6 (clustered on the p15 arm of chromosome 11) and MUC19. MUC7 and MUC9 are smaller secreted mucins that do not oligomerize and are secreted by specialized cells as monomers [3-5]. Secreted mucins comprise amino (N-t) and carboxyl terminal (C-t) regions sharing structural domains with von Willebrand (vW) factor. The central part is enriched in Pro, Thr and Ser amino acid residues forming the variable PTS domain that is O-glycosylated [4]. The *O*-glycosylation process is crucial for mucin secretion, stability, processing, and functions during both development and pathophysiological conditions [6-8]. The adjacent CYS domains are highly hydrophobic and are believed to cause the aggregation of mucins [9]. By forming disulfide bonds, the main intestinal mucin MUC2 dimerizes *via* its C-terminal cysteine-knot (CK) domain and also trimerizes *via* N-t vWD domains building a

complex molecular network [10, 11]. On the contrary MUC5AC and MUC5B are linear disulfide-linked polymers that polydisperse and that behave as random coils in solution [4]

The membrane-bound mucins are type I membrane-anchored proteins including MUC1, MUC3, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, MUC21 and MUC22 [12-14]. Typically, membrane-bound mucins contain a long extracellular domain, a hydrophobic transmembrane domain, and a short cytoplasmic tail. Analysis of the peptidic sequences of mucins allowed description of their modular organization. The PTS domain, the only domain not conserved at the genomic level, is the common feature between mucins. Membrane-bound mucins share conserved domains such as epidermal growth factor-like (EGF) or Sea urchin sperm protein Enterokinase and Agrin (SEA) domains [13, 15, 16]. Based on their structure and localization at the cell surface they were shown to act in cell-cell, cell-extracellular matrix interactions and in cell signaling.

Mucins have a cell- and tissue-specific patterns of expression profoundly altered in epithelial cancers (loss of expression, over-expression, aberrant expression, neo-expression, glycosylation alterations) [17-21]. Because of their specific pattern of expression during the different steps of tumor progression toward adenocarcinoma, mucins stay under intense investigation as both potent new biomarkers and therapeutic targets in epithelial cancers.

Numerous reviews in the literature describe the roles of mucins in relation to cancer cell behavior and cell signaling pathways associated with tumorigenesis. Among them, membrane-bound mucins MUC1 and MUC4 have been extensively studied [14, 22-25]. MUC1 and MUC4 govern both cellular differentiation and proliferation. They are also involved in metastasis and tumor proliferation. Secreted mucins MUC5B and MUC5AC and membrane bound mucins MUC13 and MUC16 have also been associated with aggressive behavior of cancer cells [26-31]. On the contrary, Muc2 is involved in the suppression of

colorectal cancer (CRC) since $Muc2^{KO}$ mice develop adenoma progress with age to invasive adenocarcinoma in the small intestine [32].

The dramatic outcome of epithelial cancers of the gastrointestinal tract is often related to a lack of efficient therapeutic tools and early diagnostic markers. Patients that are not considered eligible for surgical resection frequently benefit from chemotherapy. However, chemoresistance is a common feature of epithelial cancers. Lately, mucins have been proposed as actors of this phenomenon. In this review, we will discuss their role and the associated cellular mechanisms in chemoresistance in order to propose them as therapeutic tools and/or prognosis biomarkers of chemotherapy response.

2. Mucins and chemoresistance *in vitro*

In our laboratory and others, initial *in vitro* studies showing the relationship between mucin and chemoresistance came from colorectal carcinoma cells (HT29) stably resistant to 5-fluorouracil or methotrexate [33-35]. These cells were characterized by the overexpression of secreted mucins when they became resistant cells. This observation pointed out to the potential of mucins as actors of chemoresistance (Table I).

In breast cancer cells, the overexpression of MUC1 is involved in cell sensitivity to Herceptin® *via* the increase of the cleavage of this mucin. These cells are also resistant to paclitaxel (Taxol®), doxorubicin and cyclophosphamide, suggesting a broader involvement of membrane-bound mucins [36]. Similarly, silencing the *MUC1* or *MUC4* gene can reverse resistance to trastuzumab in HER2-positive gastric cancers [37, 38].

Xenograft tumors of estrogen receptor positive (ER)/HER2-overexpressing breast cancer cells, that are developing resistance to lapatinib and trastuzumab, harbor an increase in mucin-filled vacuoles and upregulation of several mucins including MUC4 [39] suggesting a role of MUC4 in acquired resistance to chemotherapy. MUC4 influence on chemosensitivity has

been studied in pancreatic cancer cells using gain or loss of function strategies. Several reports showed that MUC4 protects pancreatic cancer cells from gemcitabine-induced cytotoxicity [40-43]. Similar observation was made regarding another cytidine analog, the cytarabine/aracytin ARA-C [42] or 5-fluoro-uracile (unpublished data). Overexpression of rat orthologue MUC4/SMC in melanoma cells also reverts antiproliferative effect of taxol, doxorubicin, vinblastine, rhodamine-123 or 2-deoxyglucose and cell death induced by doxorubicin [44]. Finally MUC4 expression was also shown to reduce the mitochondrial damage in pancreatic cancer cells induced by the inhibition of the proteasome when treated by the boronic acid derivative bortezomib [43].

3. Mucins and clinical response to chemotherapy

3.1 Mucinous tumors

Retrospective clinical studies indicate that mucinous carcinoma or adenocarcinoma (uterine, colorectal or breast cancer), characterized by an abundant mucus gel covering the tumor, are less sensitive to neoadjuvant chemotherapies and radiotherapies. These data support that a link exist between the mucus and chemotherapeutic efficiency in the tumor context [45-48]. In advanced gastric cancer, the signet ring cell (SRC) histologic subtype, characterized by cells containing a large mucus vacuole, is an independent predictor of poor prognosis. Moreover, perioperative chemotherapy provides no benefit on survival [49]. However, the direct involvements of secreted mucins in these clinical observations, as well as the associated underlying cellular mechanisms, remain to be determined.

3.2 Non-mucinous tumors

On the contrary, a fair number of studies have shown that tumors expressing MUC1 exhibit an increased capacity to resist the effects of chemotherapeutic drugs in breast and ovarian

cancers. Frequency analysis of MUC1 expression in an important cohort of 691 breast cancer biopsies showed that both MUC1 mRNA and protein high expression was associated with lower probability of complete response to neoadjuvant chemotherapy [50]. MUC1 regulates cholesterol and fatty acid metabolism in human breast cancer. Activation of these pathways in ER(+) breast cancers predicts failure to tamoxifen treatment with higher risk for death and recurrence/distant metastasis [51].

MUC1 protein level is significantly increased in platinum resistant ovarian tumors compared to those in sensitive-group because of chromosomal amplifications of 1q21-q22 [52]. A follow-up analysis of 92 ovarian cancer patient cohort also showed that the increase of MUC1 expression is a significant risk factor for chemoresistance to taxane combined platinum-based drugs in patients with ovarian epithelial cancer [53].

4. Mucins form a physical barrier

The outstanding web formed by the secreted mucus as well as the extraordinary size of heavily glycosylated membrane-bound mucins such as MUC1, MUC4 or MUC16 (>10^6 Da) suggest that mucins are capable to limit (i) drug intracellular entrance and/or (ii) the accessibility of the plasma membrane and its tumor cell epitopes for immune recognition or antibody-based therapy (Figure 1A).

Because of the viscosity/rheology of the mucus, secreted mucins may stop poorly soluble drugs diffusion through the cell membrane. In diseases characterized by dense mucus such as chronic inflammatory cystic fibrosis, the mucus permeability is also decreased making the diffusion more difficult for therapeutics molecules [54]. Two functions are hypothesized: Either mucins act as size filters and allow the entrance of drugs smaller than mucus network holes, or they act as interaction filters via electrostatic or hydrophobic forces accordingly to their surface properties and independently of drug molecular size [55] as ionisation of the

drug was shown to influence its diffusion through mucus [56]. The size, the electric charge and the hydrophilic property of mucins molecules composing the mucus is critical for the permeability. The electronegative potential of mucins, mostly carried by sialic acids on *O*-glycosidic chains, could affect the interaction affinity of mucus [20] and can create electrostatic interactions with positively charged molecules (as amikacin or gemcitabine) decreasing their diffusion [57].

The steric hindrance of membrane-bound mucins is closely linked to the number and length of O-glycosidic chains worn by mucins. The benzyl-2-acetamido-2-deoxy-α-d-galactopyranoside (benzyl-α-GalNAc) is a reagent used to inhibit the synthesis of mucin O-glycosylation in cellular models. The inhibition of *O*-glycosylation in MUC1 expressing CAPAN-1 and HPAF-II pancreatic tumor cells resulted in significant 5-fluorouracile (5-FU) antiproliferative activity [58]. Benzyl-α-GalNAc that leads to a storage phenotype and abnormal intracellular localization of apical glycoproteins, does not alter directly mucin expression in CAPAN-1 cells since it was previously shown that MUC1 kept its normal localization [59]. Therefore, Kalra and Campbell speculated that the inhibition of mucin glycosylation may reduce the formidable mucin O-glycosylation mesh and facilitate the entrance of chemotherapeutic drug [58]. In this model, the mucin network is thought to limit the intracellular uptake of 5-FU and to attenuate its chemotherapeutic effect independently of sialic acid [60]. The mucus layer on the surface of normal epithelial cells was also shown to limit the diffusion of nutrients and small molecules depending on their size and physicochemical properties [61].

The overexpression of the high-molecular weight glycoprotein membrane-bound mucins on the target tumor cell surface can mask the surface antigens, and thereby decrease their accessibility and the cytotoxic response induced by antibody-based therapy as well as the tumor cell killing mediated by immune cells. It has been shown that overexpression of rMuc4,

MUC1 or MUC16 can create an immunosuppressive barrier by decreasing the accessibility of immune cells (as lymphokine-activated killer cell) to antigenic epitope dependent on *O*-glycosylation length [62-64]. The overexpression membrane-bound mucins block drug accessibility to therapeutic targets such as oncogenic receptors EGFR, ErbB2, VEGFR targeted by Erbitux/Cetuximab, Herceptin/trastuzumab, Avastin/Bevacizumab, respectively. The overexpression of rMuc4 in pancreatic cancer cell has been shown to decrease the recognition and thus the efficiency of the monoclonal antibody trastuzumab (Herceptin) which targets the oncogenic receptor ErbB2 [65]. A converse correlation has been established between the expression of MUC4 and Herceptin sensitivity in mammary cancer cells. The JIMT-1 cell line expressing the highest level of MUC4 is highly resistant to this monoclonal antibody [66].

5. Resistance to apoptosis

The induction of apoptosis is the main goal of all cytotoxic therapy. Programmed cell death resistance is a major cause of chemoresistance in which expression of mucins can reduce the sensitivity of cancer cells to genotoxic drugs by decreasing the apoptotic effect following DNA damages or physiologic stress (Figure 1B).

5.1. MUC1

MUC1 role as an apoptotic inhibitor in cancer cells is well-documented (Figure 2). Indeed, MUC1 decreases apoptotic mitochondrial factors release, caspase-3 activation, and subsequent apoptosis induction in CRC cells treated with the genotoxic agent cisplatine [67]. MUC1 cytoplasmic tail (MUC1-CT) inhibits the activation of a large array of intrinsic apoptotic pathways by regulating different signaling pathways such as p53, FOXO3a, c-Abl, IκB complex, FADD or Bax. MUC1-CT interacts with p53 tumor suppressor on its

responsive elements to coactivate p21 at the transcriptional level. On the contrary, MUC1 attenuates *Bax* transcription [68]. MUC1-CT also associates with Bax in the cytoplasm and the mitochondria *via* their CQC motif and BH3 domains, respectively, and blocks Bax dimerisation and subsequent cytochrome c release that normally activate the mitochondrial death pathway [69]. MUC1-CT anti-apoptotic function is also mediated *via* c-Abl sequestration within the cytoplasm and blocking of c-Abl and cytosolic 14-3-3 interaction [70]. Mutation of MUC1-CT at Tyr 60 (Y60F) disrupts the MUC1-Abl interaction inducing ARF expression and inhibiting MDM2-p53 pathway [71]. MUC1 is important for physiological activation of IκB kinase-β (IKK β) complex and sustained activation of NF-κB pathway in response to TNF-α activation [72]. In response to TNF-α, MUC1-CT also interacts with caspase-8 and Fas-associated death domain following death receptor stimulation and block the activation of apoptosis process [73]. In clear renal cell carcinoma (cRCC), overexpression of MUC1 prevents anoïkis through anti-apoptotic NF-κB nuclear localization, caspase-9 expression and the increase of Bcl_{XL}/Bax ratio [74].

Normal cellular metabolism is associated with the production of reactive oxygen species (ROS) including superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals, and nitric oxide. Increase in ROS levels can cause damage to DNA, proteins, and lipids [75, 76]. MUC1 reduces apoptosis following oxidative stress by regulating endogenous and H_2O_2-induced intracellular levels of ROS. Reciprocally, MUC1 expression is up-regulated by oxidative stress at the transcriptional level [77]. MUC1-CT also induces FOXO3a activation and attenuates the oxidative stress in colon and breast cancer cells [78]. Chronic ROS exposure leads to HIF-1α stabilization and up-regulation of downstream target genes [79]. Among them, MUC1 is directly regulated by HIF-1α in kidney cancer cells [80].

5.2. MUC4

An anti-apoptotic role has been described for MUC4 in serum-free conditions [81, 82] as well as following drug treatment with thymoquinone, an antioxidant and anti-tumoral compound found in plants [83], or with gemcitabine [40, 42]. MUC4 expression induces apoptosis *via* multiple intracellular mechanisms (Figure 3). It reduces mitochondrial cytochrome c release and activation of caspase-9 through phosphorylation of its partner HER2 and sequestration of pro-apoptotic Bad in the cytosol [40, 41]. Loss of MUC4 oncoprotein is accompanied by a blockage in the G1-early S phases [41, 84], an increased expression of the proapoptotic marker Bax and apoptotic mediator p53 as well as a decreased expression of the antiapoptotic Bcl_{XL} suggesting a higher susceptibility to apoptosis [42]. The overexpression of rMuc4 induces chemoresistance to paclitaxel, doxorubicin and cisplatin. In this case, rMuc4 expression decreases PARP and caspase-9 cleavage and modulates apoptosis [44, 85]. rMuc4 may also repress apoptosis *via* an ErbB2-dependent mechanism, in which rMuc4 potentiates the activation of ErbB2 by phosphorylation of the Y1248 leading to activation of the PI3K/Akt pathway, and *via* an ErbB2-independent mechanism that remains to be determined [86, 87]. MUC4 overexpression in ovarian cancer cells also leads to increased $CD133^+$ cell population. CD133 is a pentaspan transmembrane glycoprotein associated with chemo-resistance, radio-resistance and poor prognosis and is commonly used as stem cell marker (see §7). Additionally, $CD133^+$ cell population demonstrates significant resistance to drug-, TGF-β- and TNF-related apoptosis-inducing ligand (TRAIL)- induced apoptosis compared with $CD133^-$ cells [88, 89] suggesting that CD133 triggers resistance to apoptosis in MUC4-expressing cells via similar mechanisms.

6. Mucins and alteration of drug metabolism

In pancreatic cancer, chemoresistance has been attributed partly to the enhanced expression of multidrug resistance (MDR) genes (Figure 1C) including ATP-binding cassette transporter

genes (ABCC)1, ABCC3, ABCC5 and ABCB1 that encode MDR related proteins (MRP)-1, MRP3, MRP5 and MDR1 proteins, respectively [90]. The proteins encoded by this gene family are ATP-dependent drug efflux pumps for xenobiotic compounds with broad substrate specificity [91]. Notably, cleaved MUC1-CT directly associates and activates the ABCC1 promoter and increases (i) ABCC1 protein level in MUC1-high pancreatic cancer cells and (ii) resistance to gemcitabine and etoposide chemotherapeutic drugs [90] (Figure 2).

One way to explain modifications of cell sensitivity to nucleoside analog such as gemcitabine or 5-FU is an alteration of the actors responsible for their metabolism and more particularly nucleoside transporters. We and others have described a major role for MUC4 in resistance to gemcitabine chemotherapy [40-42] involving alteration of nucleotide metabolism. In that case, MUC4 mucin negatively regulates the hCNT1 transporter expression *via* the NF-κB pathway, pointing out to MUC4 and hCNT1 as potential new targets to ameliorate the response of pancreatic tumors to gemcitabine treatment [42].

7. Cancer stem cells

The ability to relapse after therapy is frequently dependent on a small subset of the cell population within the tumor, called cancer stem cells (CSC) or side population (SP) (Figure 1D), characterized by an extensive self-renewing capacity [92]. Some therapies including chemotherapy may provide strong selection for CSC survival and proliferation [93].

Very recently, MUC1-CT was shown to upregulate expression of breast CSC marker Aldehyde dehydrogenase 1A1 (ALDH1A1) *via* Erk1 and C/EBPβ by forming a transcriptional activating complex on the *ALDH1A1* gene promoter [94]. MUC1 was also shown to be expressed by $CD34^+$ $CD38^-$ acute myeloid leukemia (AML) cells which have been associated with leukemia stem cells (LSC) suggesting that MUC1 represents a potential target on the AML stem cell population [95].

In ovarian cancer, MUC4 overexpression leads to increased CD133-positive CSC [96] (Figure 3). Stem cell-like marker CD133 is also observed in a small subpopulation of pancreatic epithelial cells in the basal compartment in non-malignant pancreatic tissue specimens. This subpopulation also expresses MUC4 membrane-bound mucin [41]. MUC4 oncoprotein which is normally not expressed in the normal pancreas, is expressed at a high level in both the small $CD133^{+}$cell progenitor subpopulation as well as their differentiated $CD133^{-}$ progenies. Mimeault *et al* have suggested that MUC4 down-regulation can partially reverse the resistance of CD133+ initiating cells to the gemcitabine treatment [41]. This is notably important since gemcitabine treatment of pancreatic tumor xenografts leads to enrichment of cell subpopulations expressing stem cell markers such as ALDH and CD24 as well as efflux pumps such as ABCB1 and ABCG2 [97, 98]. MUC4 oncoprotein may thus represent a promising therapeutic strategy in that matter.

8. Epithelial-mesenchymal transition (EMT)

EMT is a physiological and pathological reversible biological process associated with loss of cellular polarity, decreased surface expression of epithelial markers (E-cadherin, cytokeratin-18, ZO-1) and increase mesenchymal markers expression (vimentin, N-cadherin, MMP-9, ZEB-1) (Figure 1E) [99, 100]. EMT inducers include notably transcription factors, such as Snail, Slug, twist1/2, transforming growth factor (TGFß) pathway or miRNA [101, 102]. Many reports show that EMT is a major step toward metastatic tumor progression and contributes to drug resistance and acquisition or selection of stem/progenitor-like features and ultimately recurrence [103-106].

MUC1 MUC4 and MUC16 are able to trigger the molecular process of EMT [107-110]. The mucin-induced EMT implies interaction with β-catenin that leads to cell-cell junction disruption and invasiveness. Interestingly, MUC1-CT interacts with β-catenin and

translocates to the nucleus in order to upregulate EMT initiating genes. Moreover, MUC1-CT occupies and induces the *ZEB1* promoter by a NF-κB p65-dependent mechanism. In turn ZEB1 associates with MUC1-CT and contributes to suppress miR200c expression that promotes EMT [111]. In cRCC, MUC1-CT also occupies the *Snail* promoter, modulates the binding of β-catenin and trigger EMT [112]. The cellular mechanisms underlying EMT related to MUC4 or MUC16 are not fully understood but might involve signaling pathway deregulation.

The relation between MUC4 and EMT is controversial since MUC4 was shown to suppress EMT in lung adenocarcinoma [113, 114] whereas MUC4 overexpression in ovarian cancer led to decreased expression of epithelial markers and occurrence of mesenchymal markers *via* an upregulation of Twist1, Twist2 and Snail transcription factors and FAK signaling pathway [110]. Collectively, these results suggest that MUC4 may regulate EMT in both ways depending on the cellular context. Complementary studies need to be carried out in order to elucidate these discrepancies regarding MUC4.

One may hypothesize that targeting EMT using MUC1 or MUC16 mucin as a therapeutic approach may be effective since mucins are involved in both chemoresistance and EMT that often lead to tumor recurrence. Recently, MUC1 siRNA was shown to inhibit both cell proliferation and EMT in urinary bladder cancer [115].

9. Mucins and polymorphisms associated with chemosensitivity?

As genomic sequencing cost declines rapidly, the availability of pangenomic information promotes the era of pharmacogenomics and therefore personalized medicine. The genetic diversity between different population and ethnic backgrounds might explain a high degree of variability of drug response and adverse drug reactions [116]. Frequency differences of

polymorphisms exist across different geographic regions for drug transporters or genes involved in drug metabolisms [117, 118].

Mucin single nucleotide polymorphisms (SNP), associated with various diseases including cancer, have been described [119, 120]. Moreover, the SNP distributions differ between subjects from different origins (Caucasian or Asian) [121]. Mucin genes also exhibit a high degree of polymorphisms in the VNTR domain [122]. The variation of VNTR length leads to quantitative differences of the O-glycan content and alters biophysical properties of the glycoprotein. Among MUC associated with disease, gastric cancer patients harbor a higher proportion of short MUC1 allele compared to a control population [123]. Despite these data, no direct association between mucin polymorphism (SNP or VNTR) and sensitivity to chemotherapeutic drugs has been described so far.

10. Outlook to the future: Using mucins as a therapeutic target to sensitize cancer cells to chemotherapeutic drugs or as biomarkers of chemoresistance?

Since oncomucin expression in cancer cells is linked to a higher susceptibility to apoptosis, an increased expression of multidrug resistance markers, and stem cell characteristics, this could represent a valuable therapeutic strategy to decrease tumor resistance. Because of their high molecular weight and their high sugar content, extraction and purification of native mucins for therapy is notoriously difficult [124]. Therefore, native mucins were never used concomitantly with chemotherapeutic drugs. However, mucins expression or functionality was targeted in order to sensitize cancer cells to chemotherapeutic drugs.

Peptide inhibitors targeting MUC1-CT were shown to block its interaction with NF-κB and to activate the induction of late apoptosis/necrosis in myeloma cells [125].

Silencing of MUC1 C-terminal subunit in HER2-overexpressing breast cancer cells or treatment with the penetrating peptide inhibitor, G0-203, reverses chemoresistance to trastuzumab, tamoxifen, taxol and doxorubicin (Figure 2) [126-128].

Silencing MUC1-CT is associated with (i) downregulation of HER2 phosphorylation and (ii) sensitivity to tamoxifen-induced growth inhibition and loss of clonogenic survival. HER2-positive cancer cells that are intrinsically resistant to trastuzumab became sensitive when treated with an antagonist of the cleaved form of the MUC1 protein [36]. In bladder cancer, MUC1 silencing also leads to reduction of tumor volume *in vivo* and suppresses EMT. However, the impact on chemosensitivity remains to be proven in this case [115].

Currently, phase 1 multi-center clinical trial testing G0-203 dose-escalation, safety, pharmacodynamic and pharmacokinetic in solid tumors and lymphomas is in progress (ClinicalTrials.gov identifier: NCT01279603). G0-203 also resulted in tumor regression independently of chemotherapy, in non-small cell lung cancer cells and acute myeloid leukemia [95, 129].

Numerous studies are already targeting MUC1 as an immunotherapeutic approach [130-133]. Mucins could also be used in modified vectors sensitizing the tumor to targeted chemotherapy. In OVCAR-3 ovarian cancer cells, a MUC1/let-7i chimera, combines MUC1 aptamer and let-7i miRNA, can specifically reverse chemoresistance to paclitaxel [134]. Similarly a MUC1/miR-29b chimera also reverses chemoresistance to paclitaxel [135]. However, these effects on chemoresistance are independent of MUC1 structure since the MUC1 aptamer alone does not influence the induced apoptosis.

Therapy-predictive markers are factors that prospectively identify response or resistance to a specific treatment. They allow distinguishing patients with the same histological type of malignancy that respond very differently to a specific drug [136]. In CRC, the presence of acellular mucin pools in the resected specimens of patients with a complete response to

preoperative chemoradiation is frequently reported as a marker of treatment effect. However, several reports ruled out its prognostic significance [137-139].

An ideal marker implicates non-invasive and rapid procedures of detection. Therefore, clinicians routinely use robust circulating markers in the blood or markers such as tumor-associated antigens. MUC1 (CA15-3) and MUC16 (CA125) tumor-associated antigens are commonly detected in patients' sera by standardized tests such as ELISA or immunohistochemistry [136, 140]. MUC1 and MUC16 are overexpressed in ovarian cancer [17, 141]. Budiu *et al.,* showed that increased serum MUC1 level has a prognostic value for poor clinical response and reduced overall survival in platinum-resistant or platinum-refractory ovarian cancer whereas MUC16 was not associated with clinical response [140].

In a multivariable analysis, MUC1 protein and mRNA expression were independently predictive in a breast cancer cohort [50]. CA15.3 level is also correlated with treatment response in patients undergoing chemotherapy for metastatic breast cancer [142]. Detection of (MUC1)-positive circulating tumor cells and MUC1 protein in the peripheral blood of patients with metastatic breast cancer is thus associated with higher progression-free survival (PFS) [143].

11. Conclusion

Over the past two decades, spectacular advances in targeted therapy led to improvement of treatment of a great numbers of cancer patients. These outstanding results are counterbalanced by the increased occurrence of acquired or intrinsic events of chemoresistance that limits long term success. Many evidence support the involvement of both membrane-bound and secreted mucins in diverse biological mechanims of resistance (physical barrier, apoptosis resistance, drug catabolism or exclusion, cell stemness, EMT). Understanding mechanisms of resistance involving mucins shall contribute to the development of next generation targeted therapy molecules. Alternatively, mucins will help in the prevention because of their potential as tumor biomarkers and orientate the therapeutic choice tree toward the potentially more successful therapy and more importantly avoid a more aggressive alternate therapy that is promised to failure.

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Figure legends

Figure 1: Mucins and chemoresistance in epithelial cells. (A) Secreted and membrane-bound mucins form a physical barrier blocking the accessibility of the cell to therapeutic drugs. Mucin overexpression is associated with (B) apoptosis resistance, (C) drug exclusion or reduction of drug intake, (D) cell stemness and (E) epithelial-mesenchymal transition. Cancer stem cells (CSC) belong to the small subset of cells (side population) commonly expressing CSC markers such as CD133 visualized here by flow cytometry. EMT is associated by the loss of cell polarity, decreased expression of epithelial markers and increased expression of mesenchymal markers (e.g. vimentin).

Figure 2: MUC1 overexpression and associated cellular mechanisms of chemoresistance in epithelial cancer cells.

Figure 3: MUC4 overexpression and associated cellular mechanisms of chemoresistance in epithelial cancer cells.

Table 1: Mucins and drug chemoresistance in epithelial cancer cells

Mucin	Drug	Tumor type	Refs
Membrane-bound mucins			
MUC1	5-FU/Methotrexate	CRC	[34, 35]
	5-FU	PDAC	[58, 60]
	Cisplatin	Ovarian	[52]
	Cisplatin	CRC	[67]
	Taxane/Platinum compound	Ovarian	[53]
	Trastuzumab/ Paclitaxel/Doxorubicin/ Cyclophosphamide	Breast Cancer	[36]
	Gemcitabine/Etoposide	PDAC	[90]
MUC3	Methotrexate	CRC	[34]
MUC4	5-FU	CRC	[34]
	Lapatinib/Trastuzumab	Breast Cancer	[39]
	Trastuzumab	Melanoma/Breast Cancer	[65]
	Cytarabine/Aracytin	PDAC	[42]
	Paclitaxel/Doxorubicin/ Vinblastine/Rhodamine-123/2-deoxyglucose	Melanoma	[44]
	Bortezomib	PDAC	[43]
	Gemcitabine	PDAC	[40-42]
Secreted mucins			
MUC5AC	5-FU/Methotrexate	CRC	[34, 35]
MUC5B	5-FU/Methotrexate	CRC	[34, 35]
MUC2	5-FU	CRC	[34]

Figure 1

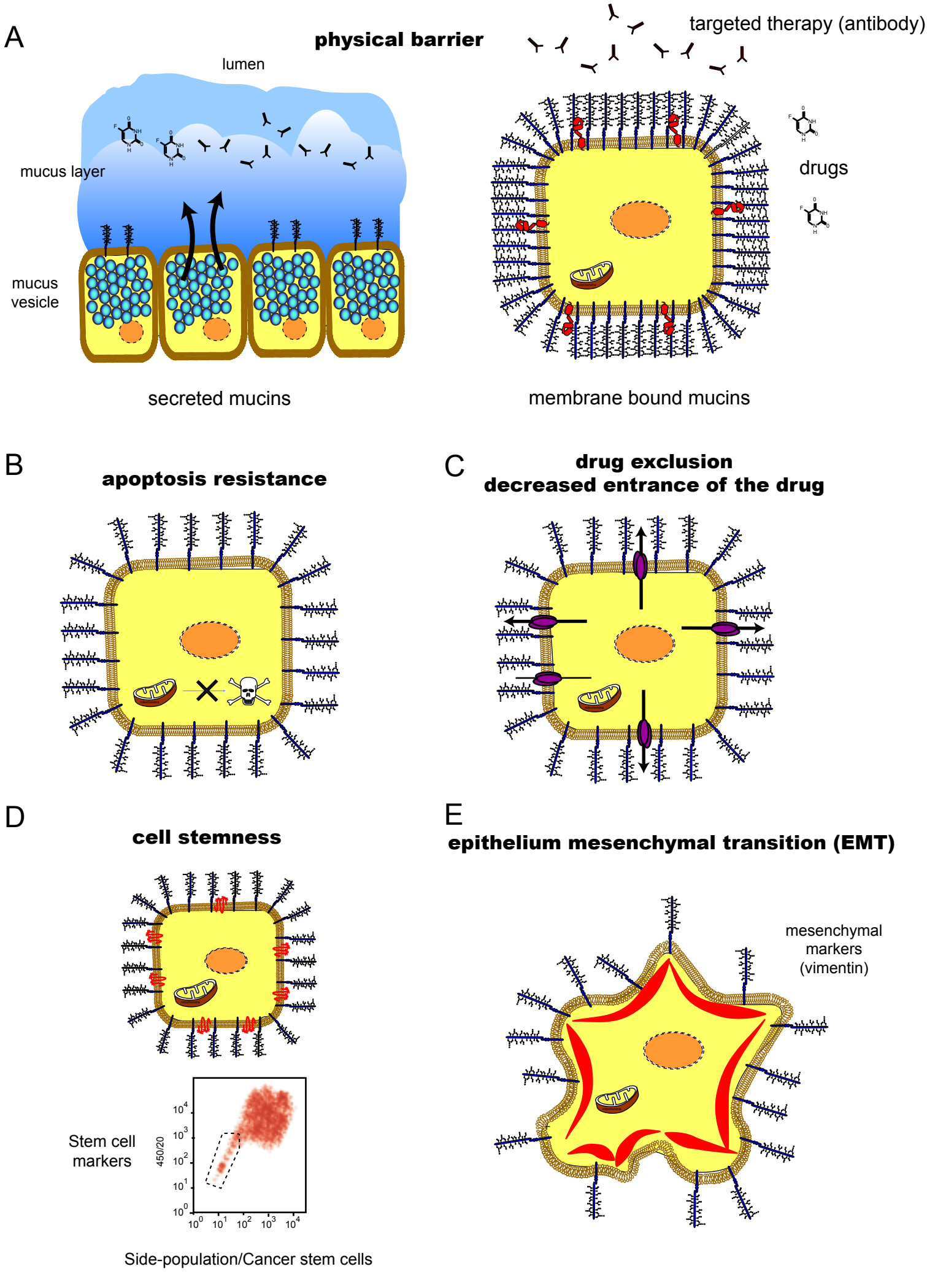
A
physical barrier
lumen
mucus layer
mucus vesicle
secreted mucins
targeted therapy (antibody)
drugs
membrane bound mucins
B
apoptosis resistance
C
drug exclusion
decreased entrance of the drug
D
cell stemness
Stem cell markers
450/20
Side-population/Cancer stem cells
E
epithelium mesenchymal transition (EMT)
mesenchymal markers (vimentin)

Figure 2

Figure 2

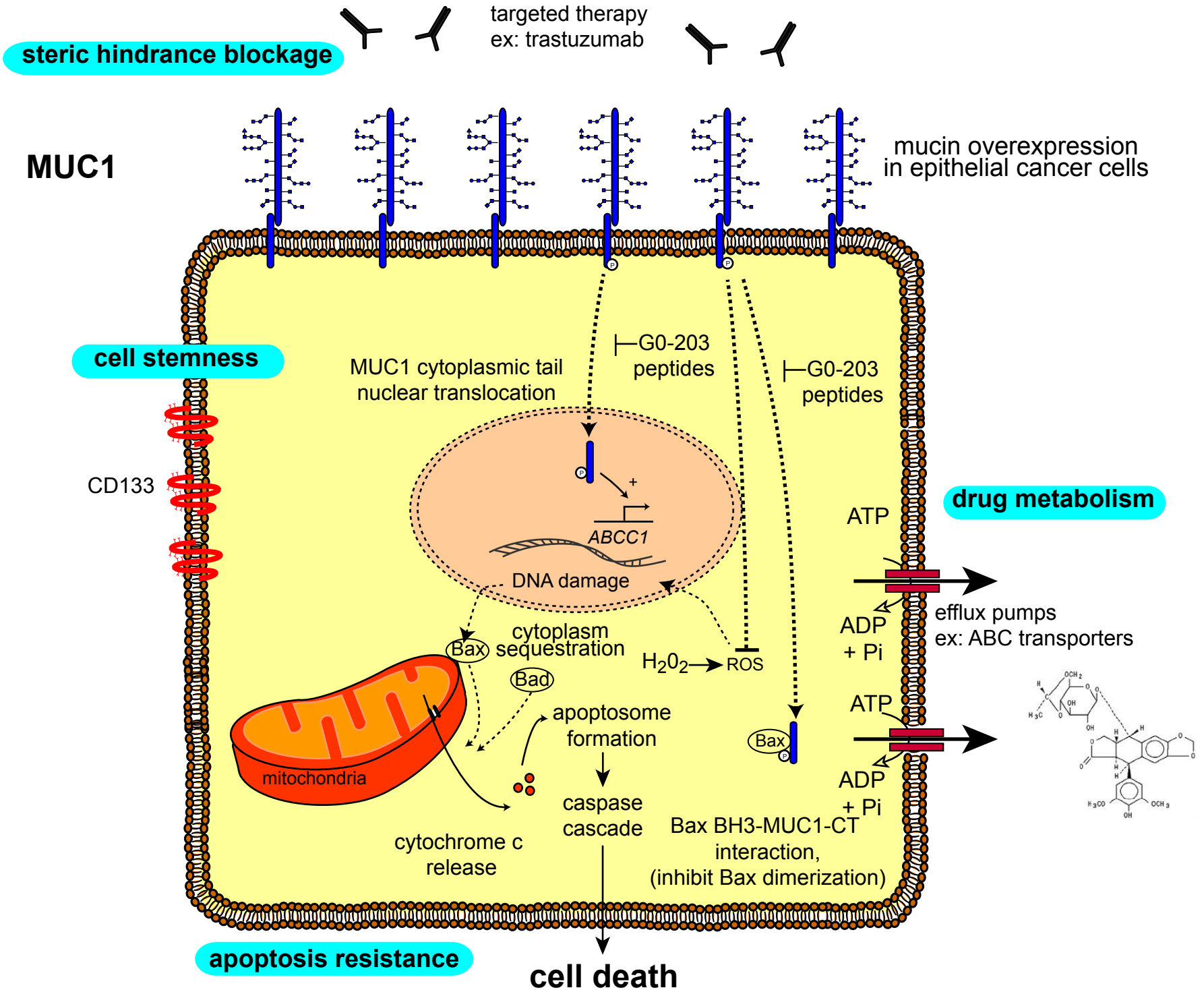

Figure 3

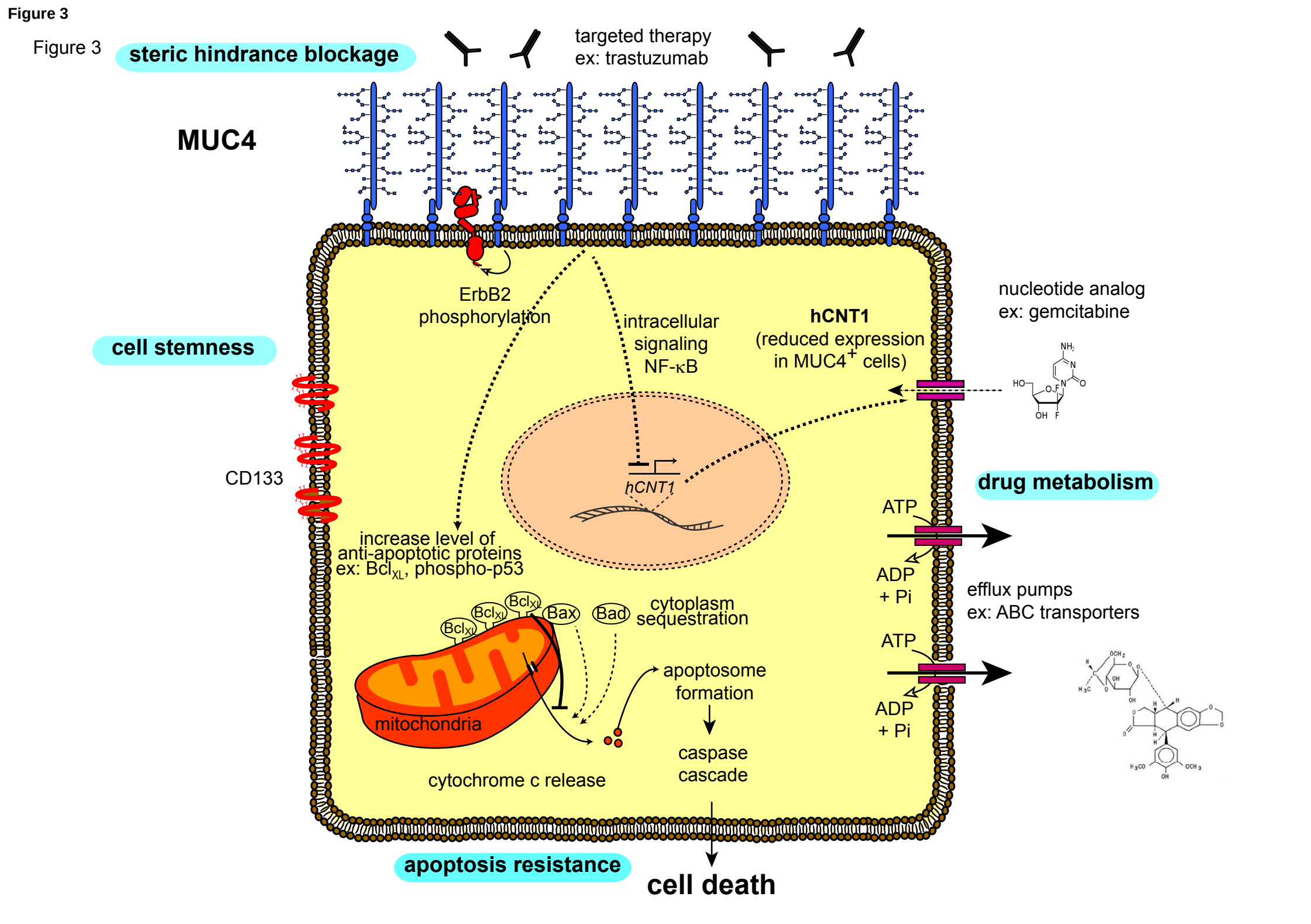
Figure 3
steric hindrance blockage
targeted therapy
ex: trastuzumab
MUC4
ErbB2
phosphorylation
intracellular
signaling
NF-κB
hCNT1
(reduced expression
in MUC4+ cells)
nucleotide analog
ex: gemcitabine
cell stemness
CD133
hCNT1
drug metabolism
ATP
ADP
+ Pi
efflux pumps
ex: ABC transporters
increase level of
anti-apoptotic proteins
ex: BclXL, phospho-p53
BclXL
Bax
Bad
cytoplasm
sequestration
apoptosome
formation
mitochondria
caspase
cascade
cytochrome c release
apoptosis resistance
cell death