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Mechanisms of cisplatin resistance and targeting of cancer stem cells: Adding glycosylation to the equation

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ABSTRACT

Cisplatin-based chemotherapeutic regimens are the most frequently used (neo)adjuvant treatments for the majority of solid tumors. While platinum-based chemotherapeutic regimens have proven effective against highly proliferative malignant tumors, significant relapse and progression rates as well as decreased overall survival are still observed. Currently, it is known that sub-populations of chemoresistant cells share biological properties with cancer stem cells (CSC), which are believed to be responsible for tumor relapse, invasion and ultimately disease dissemination through acquisition of mesenchymal cell traits. In spite of concentrated efforts devoted to decipher the mechanisms underlying CSC chemoresistance and to design targeted therapeutics to these cells, proteomics has failed to unveil molecular signatures capable of distinguishing between malignant and non-malignant stem cells. This has hampered substantial developments in this complex field. Envisaging a novel rationale for an effective therapy, the current review summarizes the main cellular and molecular mechanisms underlying cisplatin resistance and the impact of chemotherapy challenge in CSC selection and clinical outcome. It further emphasizes the growing amount of data supporting a role for protein glycosylation in drug resistance. The dynamic and context-dependent nature of protein glycosylation is also comprehensively discussed, hence highlighting its potentially important role as a biomarker of CSC. As the paradigm of cancer therapeutics shifts towards precision medicine and patient-tailored therapeutics, we bring into focus the need to introduce glycomics and glycoproteomics in holistic pan-omics models, in order to integrate diverse, multimodal and clinically relevant information towards more effective cancer therapeutics.

1. Introduction

Cisplatin (cis-diamminedichloridoplatinum(II); cis-$[Pt(NH_3)_2(Cl)_2]$) was first described by Michele Peyrone in 1845, but its structure was only determined in 1893 (Trzaska, 2005). After several years of investigation, Rosenberg realized its potential to induce tumor cells death (Rosenberg, 1973) and finally in 1978 the drug was approved by the FDA for the treatment of testicular and ovarian cancer (Trzaska, 2005). Nowadays, cisplatin-based regimens are widely used as (neo)adjuvant chemotherapy against a spectrum of solid tumors including gastric, non-small cell lung (NSCLC), head and neck, gallbladder and urinary bladder cancer. However, cisplatin treatment exhibits severe side effects including immunosuppression, renal toxicity, gastrointestinal disorders and ototoxicity (Boussios et al., 2012; Karasawa and Steyger, 2015). It may also cause gonadal suppression resulting in amenorrhea or azoospermia, partial or irreversible infertility and embryo-toxicity (Brennemann et al., 1997; Meistrich, 2009).

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Cisplatin is an alkylating agent capable of forming adducts with macromolecules, particularly with N7 atoms of purine nucleobases. This results in inter- and intra-strand DNA cross-links that bring induce cell cycle arrest mainly in the G_2/M checkpoint (Yuan et al., 2003). The inability to repair this DNA damage ultimately leads to programed cell death. However, experimental evidence revealed that other mechanisms such as the production of reactive oxygen species (ROS) and the activation of inflammatory pathways, may also contribute to the induction of apoptosis (Casares et al., 2012). Cisplatin has shown significant efficacy against rapidly proliferating tumor cells. However, despite a fairly acceptable intrinsic drug response rate, there is a 95% risk of tumor relapse in NSCLC patients. The 5-year survival rate is approximately 50% for muscle-invasive bladder cancer, (Nadal and Bellmunt, 2014) and 15–20% for ovarian cancer patients (Siddik, 2003); similar survival rates have been reported for other solid tumors. It has been hypothesized that chemotherapy may either act as a selective pressure for more aggressive cell phenotypes (Freitas et al., 2014), or that tumor cells which are less drug sensitive may acquire mutations during the course of treatment, that enable them to evade drug-induced cell death (Crea et al., 2011). The failure of cisplatin-based regimens is considered both life-threatening and a major burden to health care systems, as it requires the introduction of more expensive second-line treatments. Therefore, deciphering the mechanisms underlying this treatment failure has been a primary goal of cancer research and, in the past two decades, some of the modalities underlying anticancer drug resistance have been identified; however the implications for improving drug therapy have been limited.

Chemotherapy resistance results from a synergism of events that include tumor cell extrinsic factors (pharmacokinetic resistance and tumor microenvironment) as well as intrinsic factors, namely alterations in drug transport and metabolism, relative dormancy/slow cell cycle kinetics, efficient DNA repair systems and inhibition of apoptosis (Martin et al., 2008; Pommier et al., 2004; Raguz and Yague, 2008). In addition, some chemoresistant tumor cell clones may present self-renewal and pluri/multipotent differentiation capabilities, which are characteristics associated with cancer-stem cells (CSC; Visvader and Lindeman, 2008). Therefore, these cells constitute a small pool of CSC capable of generating more differentiated sub-populations that, during subsequent divisions, form the vast majority of the tumor bulk. The remarkable longevity of CSC also renders them more susceptible to the accumulation of genetic damage and epigenetic alterations that may ultimately promote the proliferation of heterogeneous and aggressive cell phenotypes (Muñoz et al., 2012). Some subsets of CSC can be found in poorly vascularized hypoxic tumor niches, which favor the maintenance of stem-cell characteristics, and are consequently exposed to suboptimal drug concentrations (Lin and Yun, 2010). Furthermore, these cells may undergo epithelial-to-mesenchymal transition (EMT) in response to microenvironmental stimuli, namely prolonged exposure to low oxygen levels, and may acquire the capability to invade and metastasize to regional lymph nodes and distant organs (Jiang et al., 2011). In summary, it became evident that cisplatin and other conventional chemotherapeutic drugs may ultimately contribute to the selection of a pool of slow dividing or quiescent CSC (Wang et al., 2014a,b). These cells are endowed with the capability of recapitulating tumor heterogeneity and undergo EMT, considered as one of the driving forces of cancer dissemination (Frank et al., 2010). As such, patients would greatly benefit from combined therapies including agents capable of selectively eliminating CSC. The ideal therapy should specifically recognize these cells from the tumor bulk, include means to inhibit resistance mechanisms, as well as include CSC-killing agents. However, the majority of membrane-bound CSC biomarkers known to date can also be found in normal stem- and non-malignant cells (Cojoc et al., 2015), which hampers the development of specific targeted therapeutics.

More recently, several studies have demonstrated that profound alterations in protein glycosylation that often accompany malignant transformation may also influence resistance to chemotherapy. This rather neglected mechanism of drug resistance has been often associated with impaired function of membrane-bound glycoproteins, such as ATP-binding cassette efflux transporters, due to specific alterations in their glycosylation patterns (Beers et al., 2013; Nakagawa et al., 2009). However, alterations in cell-surface protein glycosylation have also been shown to favor oncogenic signaling pathways associated with chemoresistance and CSC-like phenotypes (Dall'Olio et al., 2014; Häuselmann and Borsig, 2014; Ju et al., 2008; Pinho et al., 2012). Therefore, cancer-associated glycans constitute markers of chemoresistance and bear potential promise for the identification and therapeutic targeting of CSC.

Envisaging a rationale for an effective therapy, the present review discusses the main mechanisms of cisplatin resistance known to date, integrating key insights about the role of cancer-associated glycans. Although the current review focuses mainly on cisplatin, it is proposed here that many of these strategies mediate resistance to other drugs as well. The present paper also provides a comprehensive overview on the impact of the chemotherapeutic challenge in tumor biology, CSC selection and clinical outcome. Moreover, it aims to raise awareness for the fact that CSC harbor distinct glycosylation patterns that should be carefully explored towards the development of highly specific targeted therapeutics.

2. Overview on drug resistance mechanisms and CSC selection

Drug resistance is a multifactorial process which is based on both extrinsic and intrinsic factors in tumor cells (Raguz and Yague, 2008). Extrinsic factors such as unfavorable drug pharmacokinetics and abnormal tumor vasculature result in the delivery of suboptimal concentrations of cytotoxic agents to tumor sites (Rohwer and Cramer, 2011). Defective tumor vasculature also results in hypoxic and acidic niches that significantly modulate cell function in manners that favor chemoresistance (Wilson and Hay, 2011). Similarly, alterations in the extracellular matrix architecture and stromal cell paracrine signals have been found to influence chemotherapy outcome (Sherman-Baust et al., 2003; Tripathi et al., 2012). In addition, tumor cells may either present, or develop during the course of treatment, various mechanisms to withstand and overcome chemotherapeutic challenges (Shen et al., 2012). These mechanisms include for example: (i) Alterations in drug transport and metabolism; (ii) Enhanced DNA repair mechanisms; (iii) Alterations in cell cycle regulation; and (iv) Inhibition of apoptosis. Emerging evidences support the notion that chemoresistance, driven by the above-mentioned factors, is associated with CSC-like properties as well as the acquisition of EMT capability, thereby explaining the high relapse and progression rates presented by first-line chemotherapy agents (Cojoc et al., 2015). Based on these considerations, the following sections aim to illustrate the influence of the main tumor-associated extrinsic and intrinsic properties in chemoresistance.

2.1. The impact of the microenvironment on drug resistance

2.1.1. Tumor vasculature and hypoxia

Solid tumors often present tortuous, poorly differentiated and truncated vasculature, resulting in the delivery of suboptimal concentrations of cytotoxic drugs to certain niches (Minchinton and Tannock, 2006). This also accounts for the formation of a hypoxic