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Antibody-targeted drugs and drug resistance—Challenges and solutions

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ABSTRACT

Antibody-based therapy of various human malignancies has shown efficacy in the past 30 years and is now one of the most successful and leading strategies for targeted treatment of patients harboring hematological malignancies and solid tumors. Antibody–drug conjugates (ADCs) aim to take advantage of the affinity and specificity of monoclonal antibodies (mAbs) to selectively deliver potent cytotoxic drugs to antigen-expressing tumor cells. Key parameters for ADC include choosing the optimal components of the ADC (the antibody, the linker and the cytotoxic drug) and selecting the suitable cell-surface target antigen. Building on the success of recent FDA approval of brentuximab vedotin (Adcetris®) and ado-trastuzumab emtansine (Kadcyla®), ADCs are currently a class of drugs with a robust pipeline with clinical applications that are rapidly expanding. The more ADCs are being evaluated in preclinical models and clinical trials, the clearer are becoming the parameters and the challenges required for their therapeutic success. This rapidly growing knowledge and clinical experience are revealing novel modalities and mechanisms of resistance to ADCs, hence offering plausible solutions to such challenges. Here, we review the key parameters for designing a powerful ADC, focusing on how ADCs are addressing the challenge of multiple drug resistance (MDR) and its rational overcoming.

1. Introduction

The German physician and scientist, Paul Ehrlich, is considered the pioneer of targeted therapy as more than a century ago suggested the expression “magic bullet” in the early 1900s (Pietersz and Krauer, 1994; Adair et al., 2012). Ehrlich proposed a concept of selectively delivering cytotoxic agents to a target and also suggested the use of an antibody conjugated to diphtheria toxin (Ehrlich, 1906, 1913, 1956; Papachristou et al., 1977; Kasten, 1996). These early attempts were futile mostly due to the lack of technical knowledge in obtaining antibodies (Jaracz et al., 2005). The discovery of the hybridoma technology by Kohler and Milstein (1975), extremely expedite the progress in antibodies-based anticancer research. Early ADCs used mAb from a murine hybridoma. Unfortunately, a human anti-mouse antibody (HAMA) response which caused a rapid clearance of the ADC from the bloodstream, strike the therapeutic effect. Consequently, a recombinant DNA protocol was developed, which enabled the production of chimeric and humanized mAbs with decreased immunogenicity (Carter, 2001; Jaracz et al., 2005).

When monoclonal antibodies became available in the 1970s, in the first generation ADCs, researchers aimed to enhance the tumor specificity of clinically approved chemotherapeutic drugs with well-established mechanisms of action and known toxicity profiles, such as anti-metabolites (MTX and 5-fluorouracil), DNA cross-linkers (mitomycin C) and anti-microtubule agents (vinblastine) (Pietersz and Krauer, 1994; Casi and Neri, 2012), by linking them to monoclonal antibodies (Chari, 1998). The ADCs of the first generation encountered a number of challenges (Petersen et al., 1991) such as an insufficient potency of the effector molecule, limited expression of the antigen, internalization mechanisms of antibodies that were inefficient, the localization rate of the antibodies at the tumor in patients was too low, problematic linker stability (either the linker, supposed to be controllably labile, was too stable resulting in insufficient drug release, low potency and poor efficacy, or it was too labile, resulting in premature drug release, poor target specificity and high systemic toxicity). A severe obstacle was also the immune response resulting from the use of a murine origin or chimeric monoclonal antibodies and the generation of human anti-murine antibodies (HAMA) prevented repeated cycles of therapy (Chari, 2008).

Lessons learned from these initial attempts led to an understanding that the success of targeted delivery approaches depends upon three components: the characteristic of the antibody, the

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potency of the drug and the method of linkage. This realization led to a new generation of ADCs (Chari, 2008; Perez et al., 2014). Further research and developments led to the first ADC to gain US Food and Drug Administration (FDA) approval in 2000, Mylotarg®, gemtuzumab ozogamicin (Linenberger et al., 2001; Sievers et al., 2001b). Despite initially encouraging clinical results, Mylotarg® was withdrawn from the market a decade later owing to a lack of improvement in overall survival. Although with the market withdrawal, the principal of highly potent armed antibodies for cancer therapy has opened up the antibody domain for a new and effective therapeutic modalities (Adair et al., 2012). With nearly 30 additional ADCs currently in clinical development, the potential of this new therapeutic class might finally be coming to fruition (Mullard, 2013).

2. ADC structure

2.1. Designing an ADC

ADC is a sophisticated delivery system for anticancer cytotoxic drugs. Deep understanding of the cancer biology, finding the best target antigen for a specific tumor type and a proper choice of the aforementioned three components of the ADC (mAb, linker and payload) is the first step toward successful development of an ADC (Jaracz et al., 2005; Carter and Senter, 2008). ADCs acting by sending drugs to specific cellular targets using an antibody that specifically recognize an antigen which is unique to the target cells, a potent cytotoxic small molecule and a chemical linker that holds both components together, allowing spatial and temporal control over release of the active free drug. When ADC reaches a cell expressing the antigen that is recognized by the targeting mAb, it binds and internalized via endocytosis. After the fusion of the endocytic vesicle to the lysosome, the ADC is degraded and the drug diffuses through the lysosomal membrane to access targets ultimately inducing cell death (Drake and Rabuka, 2013) (see Fig. 1).

While trying to get to the target cell in a tumor, the ADC is exposed to different conditions with each step bringing its unique challenges: when circulating in the plasma, the ADC must behave as a naked antibody. The linker must be stable to prevent premature drug release and damage to healthy tissues. It is necessary that the chemical conjugation process used to link the cytotoxic agent will not disturb the antigen binding affinity and specificity of the mAb component. An intracellular concentration of the drug must be effective even though the internalization process is usually inefficient and the number of antigen targets is often limited. Precise linker cleavage, leading to release of the original and active cytotoxic drug inside the tumor cell is essential and the potency of the drug must enable killing of the tumor cell even at low concentrations. An important difference between the 1st to later generation ADCs is that the possibility of using compounds that were too toxic as a stand-alone chemotherapy agent as suitable candidates for ADC payloads has been realized (Ducry and Stump, 2010; Teicher and Chari, 2011).

2.2. ADC's target antigens

An antigen is a molecule or molecular fragment, usually a protein, characterized by its ability to be bound at the antigen-binding site of an antibody. The mAb component of the ADC binds to a target antigen which is a tumor-associated antigen on the surface of a tumor cell, and must be internalized to deliver the cytotoxic drug to target into tumor cells (Jaracz et al., 2005; Sanderson et al., 2005; Chari, 2008). Ideally, the target antigen should be abundant and accessible and should be expressed homogeneously, consistently and exclusively on the surface of cancer cells. Antigen secretion or shedding should be minimal to prevent antibody binding to the antigen in the circulation and good internalization is also desirable, the uptake of the drug and the release in the tumor are largely affected by the rate and extent of internalization (Chari, 2008; Thurber et al., 2008; Alley et al., 2009; Teicher and Chari, 2011; Scott et al., 2012). Although preclinical studies have shown that higher antigen expression levels leads to a greater ADC potency in cell culture, later observations suggest that even antigen targets with lower copy numbers can be effective. It can be concluded that ADCs are effective over a wide range of antigen expression levels when targeted with a sufficiently potent ADC (Mao et al., 2004). The need of an antigen which is homogeneous and involved in solid tumor penetration may suggest that ADCs will be more effective for treating lymphoid tumors but, several solid tumor targets are also being tested (Polson et al., 2011; Teicher and Chari, 2011). Cell surface targets for therapeutic antibodies in oncology are not strictly tumor-specific and usually they may be expressed on cells in other tissues, which may lead to unwanted side effects. Considerable effort has recently been invested in identifying new antigen targets. Different databases such as serological, genomic, proteomic and bioinformatics have been used to identify potential new antigens and receptors that are overexpressed in tumor cell populations or that are linked to gene mutations identified as driving cancer cell proliferation (Van den Eynde and Scott, 1998; Weiner et al., 2010).

2.3. The antibody component

The most common targeting element for an ADC is an antibody although any molecule with high affinity for a tumor-associated cell surface antigen (peptides, vitamins such as folic acid, fatty acids, hormones and growth factors) may also be considered as the targeting element for an ADC (Jaracz et al., 2005; Adair et al., 2012). Antibodies are large proteins with the average molecular weight of 150 kDa and are important therapeutic agent for cancer. The successful development of candidate antibodies for the clinic involves a complex process of scientific and preclinical evaluations, informed by deep understanding of cancer biology and the properties of antibodies in vivo. Characterization of the antibody includes chemical and physical properties, detailed analysis of antigen expression, study of signaling pathways and antibody distribution. It is clear that antibodies possess several clinically relevant mechanisms of action such as manipulating tumor related signaling and promote the induction of antitumor immune responses by activating or inhibiting molecules of the immune system (Wu and Senter, 2005; Scott et al., 2007; Deckert, 2009; Weiner et al., 2010). MAbs can be used as single agents for treating cancer through binding to cancer target antigen and induction of an immunological response against the target cell. There are now more than 30 FDA approved antibodies with a little over 50% of them approved for the treatment of cancer. These include Rituxan (rituximab) for B-cell lymphomas, Herceptin (trastuzumab) for breast cancer, Campath (alemtuzumab) for certain leukemias, Erbitux (cetuximab), Vectibix (panitumumab), and Avastin (bevacizumab) for colorectal cancers. MAbs are extremely discriminating for their targets but sometimes therapeutically ineffective on their own due to the limited efficacy since they only display modest cell killing activity. Therefore they are often used only in combination with anticancer drugs, which lack the selectivity property. Because antibodies may have limited therapeutic activity, emphasis is also redirected on using antibodies as delivery vehicles for cytotoxic agents yielding highly specific ADCs as well as radioimmunoconjugates (RITs) and immunotoxins (Hertler and Frankel, 1989; Burke et al., 2002; Ricart and Tolcher, 2007; Carter and Senter, 2008; Chari, 2008; Rohrer, 2008).