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Antibody mediated CDCP1 degradation as mode of action for cancer targeted therapy

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ABSTRACT

CUB-domain-containing-protein-1 (CDCP1) is an integral membrane protein whose expression is up-regulated in various cancer types. Although high CDCP1 expression has been correlated with poor prognosis in lung, breast, pancreas, and renal cancer, its functional role in tumor formation or progression is incompletely understood. So far it has remained unclear, whether CDCP1 is a useful target for antibody therapy of cancer and what could be a desired mode of action for a therapeutically useful antibody. To shed light on these questions, we have investigated the cellular effects of a therapeutic antibody candidate (RG7287). In focus formation assays, prolonged RG7287 treatment prevented the loss of contact inhibition caused by co-transformation of NIH3T3 cells with CDCP1 and Src. In a xenograft study, MCF7 cells stably overexpressing CDCP1 reached the predefined tumor volume faster than the parental MCF7 cells lacking endogenous CDCP1. This tumor growth advantage was abolished by RG7287 treatment. *In vitro*, RG7287 induced rapid tyrosine phosphorylation of CDCP1 by Src, which was accompanied by translocation of CDCP1 to a Triton X-100 insoluble fraction of the plasma membrane. Triggering these effects required bivalency of the antibody suggesting that it involves CDCP1 dimerization or clustering. However, this initial activation of CDCP1 was only transient and prolonged RG7287 treatment induced internalization and down-regulation of CDCP1 in different cancer cell lines. Antibody stimulated CDCP1 degradation required Src activity and was proteasome dependent. Also in three different xenograft models with endogenous CDCP1 expression RG7287 treatment resulted in significant tumor growth inhibition concomitant with substantially reduced CDCP1 levels as judged by immunohistochemistry and Western blotting. Thus, despite transiently activating CDCP1 signaling, the RG7287 antibody has a therapeutically useful mode of action.

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1. Introduction

CUB-domain-containing-protein-1 (CDCP1) is a highly glycosylated, single pass type I transmembrane protein of 140 kD originally found due to its up-regulation and tyrosine phosphorylation in breast, colorectal, and lung cancer (Hooper et al., 2003; Ikeda et al., 2009, 2006; Scherl-Mostageer et al., 2001). In an analysis of 200 lung adenocarcinoma patients, high expression of CDCP1 correlated with higher relapse rate, poor prognosis, and lymph node metastasis (Ikeda et al., 2009). A study on pancreatic cancer also found that patients with high CDCP1 expression levels had a lower overall survival rate (Miyazawa et al., 2010). For renal clear cell carcinoma, CDCP1 up-regulation was reported to occur in about 33% of 230 cases analyzed by immunohistochemistry (Awakura et al., 2008). Moreover, high CDCP1 levels correlated with indicators of advancing disease and predicted a poor prognosis during a median follow-up time of 45 months. The molecular basis of such correlations is presently unclear, but could be related to the fact that CDCP1 has been found to promote cell migration, invasion, and tumor cell dissemination (Deryugina et al., 2009; Uekita et al., 2008). In agreement with this, a functional role of CDCP1 in mediating anoikis-resistance (Uekita et al., 2007) and degradation of extracellular matrix (Miyazawa et al., 2010) has been reported. However, the poor prognosis of cancer patients with high CDCP1 expression levels could also be related to a higher proliferative capacity of their tumors. In a small study with 25 breast cancer patients, Ikeda et al. (2006) found that the CDCP1-high cases also had higher levels of the proliferation-associated Ki67 antigen than the CDCP1-low cases.

CDCP1 consists of a large extracellular domain (ECD), which contains three CUB (Complement C1r/C1s, Uegf, Bmp1) domains, and a short intracellular portion (Scherl-Mostageer et al., 2001). Little is known about the functional role of the ECD of CDCP1. The intracellular domain of CDCP1 has five potential tyrosine phosphorylation sites. Tyrosine734 (Y) of CDCP1 is the main phosphorylation site for Src family kinases (SFKs) (Brown et al., 2004). Phosphorylation of this site enables SH2 domain mediated SFK binding to CDCP1, which promotes further phosphorylation at other tyrosine residues (e.g. Y743 and Y762) creating docking sites for other interacting proteins like PKCδ (Benes et al., 2005). Moreover, tyrosine phosphorylation of Y734 provides a scaffold for Src-mediated phosphorylation of PKCδ and this is required for the migration and invasion effects of CDCP1 (Miyazawa et al., 2010). Overall our understanding of downstream signaling events of CDCP1 is incomplete.

The physiological and pathophysiological stimuli that trigger tyrosine phosphorylation of CDCP1 in normal and cancer cells, respectively, are also poorly understood. CDCP1 seems to be part of an outside-in signaling mechanism triggered by detachment and this involves clustering of CDCP1 in special membrane subdomains. Alvares et al. (2008) found that a certain activating anti-CDCP1 antibody (ActGp140 mAb) can mimic this process. Addition of this antibody resulted in translocation of CDCP1 to the detergent resistant membrane fraction (DRM), and association with lipid raft components. The resulting close proximity to SFKs within lipid rafts appears to promote CDCP1 phosphorylation. However, besides antibody ligation and translocation of CDCP1 to DRMs, a parallel signal from cell adhesion is necessary, since ActGp140 mAb treatment of cells in suspension failed to induce CDCP1 phosphorylation. Moreover, not all anti-CDCP1 antibodies induce its phosphorylation (Alvares et al., 2008). The fact that there are activating and non-activating anti-CDCP1 antibodies indicates that clustering induced phosphorylation has certain spatial or conformational requirements.

Whether CDCP1 is a useful target for antibody therapy of cancer has remained unclear due to the incomplete understanding of its functional role in tumorigenesis and progression as well as the unclear molecular details of its activation and downstream signaling. Moreover, some anti-CDCP1 antibodies can promote its phosphorylation, and thus could potentially promote tumor growth and survival.

In this study, we investigated the mode of action of RG7287, an activating anti-CDCP1 antibody, and show that it is a therapeutically useful antibody. In focus formation assays, RG7287 prevents loss of contact inhibition induced by the coexpression of Src and CDCP1. Additionally, in a time to event study mice that were inoculated with MCF7 cells overexpressing CDCP1 reached a predefined tumor volume faster than parental MCF7 cells, and more importantly mice bearing tumors of the CDCP1 overexpressing MCF7 cells that were treated with RG7287 reached the predefined tumor volume concurrently with the tumors of the parental MCF7 cells. *In vitro*, addition of RG7287 triggered a rapid increase in CDCP1 associated Src activity, translocation of CDCP1 to lipid rafts, and concomitantly CDCP1 phosphorylation. We show here that Src activity is required not only for CDCP1 phosphorylation, but also for its translocation to DRMs. Prolonged RG7287 treatment resulted in Src- and proteasome-dependent down modulation of CDCP1. Moreover, RG7287 inhibited tumor growth in three mouse xenograft models *in vivo*. We suggest that this down-regulation of CDCP1 is the underlying mode of action by which the RG7287 antibody showed efficacy.

2. Materials and methods

2.1. Cell culture, expression vectors, and antibodies

NCI-H322M (NCI) and MCF7 (NCI) cells were grown in RPMI1640 medium, NIH-3T3 and GP + E86 in DMEM, containing 2 mM Glutamine and 10% FCS (Invitrogen) at 37 °C with 5% CO_2.

CDCP1 and Src cDNAs were cloned into the pLXSN retroviral expression vector (Clontech). CDCP1 cloned into pcDNA3.1 was used to stably express CDCP1 in MCF7 cells. Focus formation assays were performed as described before (Kapp et al., 2007).

Generation of the original mouse CDCP1 antibody, RG7287, has been previously described (Buhring et al., 2004). This antibody and its humanized version bind huCDCP1 with single digit nanomolar affinity (1.2×10^{-9}) as measured by surface plasmon resonance. The Fab fragment of RG7287 was prepared by papain cleavage. Phospho-CDCP1 was detected with a phospho-specific rabbit monoclonal anti-CDCP1 antibody raised against a peptide containing phosphorylated Y734. CDCP1, Src,