Tetraspanin CD151 as a target for antibody-based cancer immunotherapy

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Abstract
CD151 is a plasma membrane protein belonging to the tetraspanin superfamily which is expressed on normal cells such as endothelial cells and platelets and frequently overexpressed on cancer cells. It is known to be functionally linked to cancer metastasis. In humans, increased expression of CD151 is indicative of a poor prognosis in different cancer types. Whereas its mechanism of action remains obscure, CD151 was shown to regulate cell motility and adhesion through association with laminin-binding integrins such as a3b1 or a6b4. Several anti-CD151 mAbs (monoclonal antibodies) have been shown to display anti-metastatic activity in vivo. Inhibition of metastasis was not attributed to any effect of these mAbs on tumour cell growth, but was essentially attributed to inhibition of cell motility. We have generated anti-CD151 mAbs which can inhibit the tumoral growth in different xenograft cancer models. As expected, these mAbs were also able to inhibit metastasis in orthotopic cancer models. These data suggest that CD151 could function at multiple cancer stages, including not only metastasis cascade steps, but also earlier steps of primary tumour growth, thus reinforcing the interest of this innovative target in oncology. mAbs targeting CD151 may be of significant interest for cancer biotherapy.

Introduction
Tetraspanins are a family of membrane glycoproteins, with more than 30 members in mammals [1,2]. They are 20–30% homologous and contain highly conserved cysteine residues, indicating conserved tertiary structure. They are characterized by four transmembrane domains delimiting two extracellular regions of unequal sizes, a small extracellular loop containing 20–30 amino acids and a large extracellular loop containing 70–150 amino acids. Tetraspanins are involved in cell motility, adhesion, proliferation and differentiation, and also in signal transduction. One of their outstanding properties is their ability to form a network of multimolecular complexes at the cell surface, called the ‘tetraspanin web’ or TEM (tetraspanin-enriched microdomain), in which numerous proteins are included. Not only do tetraspanins associate with each other, but also they associate with many other proteins such as integrins, Ig superfamily proteins and growth factor receptors [2–4]. Several tetraspanin superfamily members are linked to tumorigenesis. Among these tetraspanins, some members, including CD9, CD82 and CD63, are tumour suppressors [5–8], whereas other members, such as CD151 and CO-029 (Tspan8), have been identified as promoters of metastasis [9,10].

The tetraspanin CD151, also known as PETA-3 (platelet-endothelial cell tetraspan antigen 3), SFA-1 (SF-HT activated gene 1) or Tspan24, is widely expressed in several cell types, including epithelial, endothelial, muscle and haemopoietic cells [11]. CD151 gene and protein expression has been investigated in tumour tissues from lung [12], colorectal [13,14], prostate [15], pancreatic [16], breast [17,18] and hepatocellular [19] cancer patients. Elevated expression was observed in patients with advanced disease, and higher levels of expression were found retrospectively to be associated with poorer prognosis. The function of CD151 is not well understood, but it has been shown to interact with different membrane and intracellular proteins, and thus to be implicated in different transmembrane signalling pathways and cell functions. CD151 associates strongly with the laminin-binding integrins a3b1, a6b1 and a6b4 [20,21]. Thus it was shown further to modulate integrin ligand binding and signalling [22,23] and regulate cell motility [24]. This specific association requires the Q194RD196 site located in the large extracellular loop [25]. The contribution of CD151 to adhesion-dependent signalling might also be linked to its ability to recruit signalling enzymes into the integrin complexes, such as type II PI4K (phosphoinositide 4-kinase) [20,26] or conventional PKCs (protein kinases C) [27]. CD151 has been proposed to be a molecular linker between laminin-binding integrins and growth factor receptors such as EGFR (epidermal growth factor receptor) and c-Met [17,28,29], and also to function as a positive regulator of the TGFβ (transforming growth factor β) signalling pathway [30]. In vitro inhibition of CD151 expression by siRNA (short interfering RNA) was also shown to...
Table 1 | Comparison of the in vivo and in vitro properties of different anti-CD151 antibodies described in the literature

<table>
<thead>
<tr>
<th>mAb</th>
<th>In vivo anti-tumoral activity</th>
<th>In vitro activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14A2.H1</td>
<td>n.d.</td>
<td>↑ Platelet aggregation</td>
<td>[38,39]</td>
</tr>
<tr>
<td>11B1.64</td>
<td>n.d.</td>
<td>Homotypic aggregation, platelet agonist</td>
<td>[11,40]</td>
</tr>
<tr>
<td>TS151R</td>
<td>n.d.</td>
<td>Directed at the integrin interaction site</td>
<td>[41]</td>
</tr>
<tr>
<td>TS151</td>
<td>n.d.</td>
<td>Non-restricted, immunoprecipitation/Western blot studies</td>
<td>[42]</td>
</tr>
<tr>
<td>BC3</td>
<td>n.d.</td>
<td>↓ Adhesion (laminin)</td>
<td>[22]</td>
</tr>
<tr>
<td>50-6</td>
<td>↓ Metastasis</td>
<td>↓ Invasion, ↓ migration, ↓ angiogenesis</td>
<td>[43]</td>
</tr>
<tr>
<td>SFA1.2B4</td>
<td>↓ Metastasis</td>
<td>↓ Migration</td>
<td>[10]</td>
</tr>
<tr>
<td>1A5</td>
<td>↓ Metastasis</td>
<td>↓ Migration, ↑ adhesion (matrix)</td>
<td>[44]</td>
</tr>
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Anti-tumoral activity of anti-CD151 antibodies

The concept of targeting tetraspanins with mAbs (monoclonal antibodies) to treat cancer was introduced concomitantly with their identification. The first anti-tetraspanin mAb with an antiproliferative effect was directed at CD81, formerly called TAPA-1 (target of antiproliferative antibody 1) [35]. Other anti-tetraspanin mAbs have been described with anti-tumour activity in pre-clinical animal models, such as anti-CD37 or anti-CD9 mAbs [6,36], but only a few of them were tested clinically. Today, the most promising therapeutic anti-tetraspanin agent in the oncology field is targeting CD37: TRU-016, under development by Trubion Pharmaceuticals Inc. and Facet Biotech Corp., is a humanized single-chain anti-CD37–Fc fusion molecule for the treatment of B-cell malignancies [37]. Well tolerated in a Phase I study, TRU-016 is currently being evaluated in Phase II in refractory or relapsed patients with chronic lymphocytic leukaemia. Pre-clinical studies demonstrated that it is able to induce ADCC (antibody-dependent cell cytotoxicity) and apoptosis in CD37-positive B-cells.

CD151 was originally identified in platelets and endothelial cells by using a mAb raised against human acute myeloid leukaemia cells, called 14A2.H1 [38]. Other anti-CD151 mAbs have since been generated and used to help us to understand the function of CD151 (Table 1). Some of these mAbs were shown to display different patterns of binding to cells and tissues, depending on their ability or not to recognize CD151 when it is associated with integrins [45]. These differences were confirmed by CD151-integrin α3/β1 co-immunoprecipitation and epitope mapping studies [46]. For example, mAb TS151 is able to strongly co-precipitate integrins α3 and β1 from cell lysates, whereas mAbs TS151r and 14A2.H1 show very weak co-precipitating activities owing to their high specificity for the site of interaction with integrins. Their epitope was precisely determined using a series of human CD151 mutants incorporating substitutions at amino acid residues which are not conserved between human and mouse CD151. The majority of the mAbs studied are specifically directed at amino acids located in the variable region of EC2 (extracellular loop 2) (Figure 1). Two sites of reactivity including the amino acids Leu193 and Gln194 were shown to be involved in the stable association with integrin α3β1. Table 1 summarizes the in vitro and in vivo properties of some of these mAbs. CD151 is primarily implicated in cell motility, since three mAbs were shown not only to inhibit in vitro the migration and invasion of tumoral cells, but also to inhibit metastasis in different animal models [10,43,44].

Whereas the depletion of CD151 inhibited the growth of breast cancer cells in immunocompromised animals [17,18], the same type of effect was not observed with any of the anti-metastatic mAbs described above. To identify a new anti-CD151 mAb exhibiting both anti-metastatic and anti-proliferative activities, we have generated mAbs by immunizing mice with human CD151-overexpressing cells, and evaluated them further in different xenograft animal models. Several mAbs were selected for their ability to induce a strong inhibitory effect on the growth of primary tumours [47]. To determine whether these mAbs could also have an effect on tumoral cell dissemination and lifespan, their antitumoral activity was evaluated further in orthotopic animal models. Some of these mAbs were also able to induce a significant survival increase in the treated mice. These results confirm that CD151 can be involved in the growth of tumoral cells in vivo, and show for the first time that an anti-CD151 antibody can also act at an early stage of tumorigenesis.

CD151 function and mechanism of action of anti-CD151 mAbs

CD151 is known to be a promoter of metastasis. The first evidence came from studies showing that mAbs directed at CD151 can inhibit metastasis formation in vivo [10,43] (Table 1). In addition, several reports have correlated elevated
CD151 expression with tumour progression, metastatic propensity and lower survival of cancer patients [12,15,19].

Mice deficient in CD151 expression were successfully produced to try to address the in vivo functions of CD151. Depending on the genetic background used, these CD151-null mice were viable and fertile, with no developmental defects and only minor abnormalities [48], or showed kidney defects, with abnormalities of tubular and glomerular basement membranes [49,50]. More information on CD151 functions can be deduced from in vitro experiments with CD151-null and CD151-overexpressing cells. It is now well established that CD151 plays a critical role in integrin-dependent cell motility and adhesion. Silencing of CD151 was indeed shown to inhibit migration and invasion of different cell types in vitro [24,51], whereas its overexpression enhances their migration and invasion [10,43]. The CD151 depletion can directly affect integrin-mediated intracellular signalling in different cell types, by decreasing the phosphorylation of integrin effectors such as FAK (focal adhesion kinase), Src, p130Cas, Rac1 or Lck [17,23]. Moreover, RNAi (RNA interference)-mediated silencing of CD151 expression in cancer cells was shown to impair not only HGF (hepatocyte growth factor)-stimulated migration and morphogenesis [28,52], but also HGF-driven proliferation, anchorage-independent growth and survival [28,29]. These results strongly suggest that CD151 could also be involved in the regulation of downstream pathways of growth factor/growth factor receptor systems. Furthermore, CD151 was also shown to form functional complexes with c-Met, the membrane receptor through which HGF exerts its cell-proliferative effect, in different tumoral cells [28,29,52], and to regulate intracellular c-Met signalling pathways such as MAPK (mitogen-activated protein kinase) activation via recruitment of Grb2 (growth-factor-receptor-bound protein 2) [29]. In vivo studies have recently confirmed the implication of CD151 in tumoral growth. Indeed, CD151 ablation notably delays tumour progression in different mouse xenograft cancer models [17,18,29]. Mechanistically, CD151 depletion was shown to be able to disrupt integrin-mediated activation of EGFR and c-Met. Altogether, these studies suggest a new role of CD151 as a membrane-associated scaffold for optimization of tyrosine kinase receptor–integrin cross-talk at the cell surface.

Different mechanisms of action can be hypothesized for anti-CD151 mAbs (Figure 2). First, they could induce antagonistic effects, such as by blocking lateral interactions with key partners, such as integrins α3β1 and α6β4, leading to their modulation. Such an effect was described for mAb 8C3, which is able to dissociate CD151 from integrin α3β1 and thereby to attenuate the binding activity of integrin α3β1 to laminin 10/11 [22]. Cell proliferation and tumoral growth could be inhibited directly by this integrin.
Figure 2 | Potential mechanisms of action of anti-CD151 mAbs

(1) Antagonistic effects. (2) Agonistic effects. (3) ADCC. EMT, epithelial–mesenchymal transition; RTK, receptor tyrosine kinase.

activity modulation and also indirectly by disrupting the collaboration of integrins with growth factor receptors such as c-Met or EGFR (Figure 2). Secondly, another potential mode of action is that mAbs could exert an agonist effect by activating partner functions or inducing tetraspanin partner clustering (Figure 2). For example, the strong inhibitory effect on metastasis observed with mAb 1A5 (Table 1) was due to the stimulation of cell matrix adhesion, probably through integrin activation, which prevented tumoral cell detachment from the primary tumour site and inhibited invasion of the stroma surrounding the tumour [44]. Activation of the ability of CD151 to stabilize cell–cell contacts might represent another mode of action of anti-metastatic mAbs. CD151 can interact with cell–cell adhesion proteins and regulate the dynamic stability of carcinoma cell–cell contacts [34], by organizing multimolecular complexes containing PTPμ (protein tyrosine phosphatase μ), E-cadherin and β-catenin [33,34]. Thus the in vivo use of an anti-CD151 mAb could induce cell–cell junctions and favour homotypic cell adhesion, as described previously for mAb 11B1.G4 [40], by clustering cell junction proteins such as cadherins into TEMs. The increase in cell–cell contacts might then block the epithelial–mesenchymal transition, which constitutes one of the key steps in the metastasis process. E-cadherin clustering and homophilic binding can also induce growth inhibition by a β-catenin-dependent mechanism that inhibits selective signalling functions of growth factor receptors such as EGFR [53], as well as by reducing the level of active forms of Rho family proteins, especially RhoA and Cdc42 [54]. CD151 itself was also shown to participate in these Rho-suppressive functions [34]. The anti-proliferative signals induced by EGFR–cadherin complexes can be opposed to the pro-proliferative signals generated by ligand-activated integrin/RTKs (receptor tyrosine kinases) [55]. Thirdly, an effect on angiogenesis can also be expected since (i) CD151 is expressed on human endothelial cells, (ii) pathological angiogenesis is impaired in CD151-null mice, and (iii) CD151-null endothelial cells show marked alterations in different in vitro assays relevant to angiogenesis [56].

The last potential mode of action is linked to the antibody effector functions (Figure 2) such as ADCC and CDC (complement-dependent cytotoxicity) [57]. Induction of these cytolytic effector functions is highly dependent on the antibody isotype. Among the different human IgG isotypes used in therapeutic antibodies on the market and in clinical development, IgG1 antibodies are the most potent in mediating both ADCC and CDC.

Concluding remarks and prospects for future clinical use of anti-CD151 antibodies

Many experimental data suggest that anti-CD151 mAbs could block cancer at multiple stages, including tumour
growth, angiogenesis and metastasis, by affecting ligand binding or downstream processing of associated molecules, such as integrins and growth factor receptors. However, the widespread distribution of CD151 on numerous cells and tissues could constitute an obstacle to its targeting. CD151 has indeed a broad distribution in normal tissues [11]. It is expressed in epithelial and endothelial cells, with predominant localization in hemidesmosomes and basal structures in these cells, as well as in some blood cells such as monocytes and platelets. Patients with a single nucleotide insertion in the CD151 gene, leading to a translated protein lacking most of the large extracellular loop, have been identified [58]. The major consequences of this mutation are restricted prethelial epidermolysis bullosa and hereditary nephritis, pathology which can be compared with the renal defects observed in CD151−/− mice [49,50]. The probability of observing such defects in human after treatment with anti-CD151 mAbs is low, but side effects cannot be excluded. For example, considering the abundant expression of CD151 on platelets and endothelial cells, its targeting could affect physiological functions of these normal cells. In addition, the choice of the antibody isotype can be critical. For example, the use of IgG4 or non-glycosylated IgG1 formats, which are known to have no effector functions, could also allow the restriction of the antibody activity to tumoral cells without affecting normal cells. According to the guidelines recently introduced by the European Medicines Agency (EMEA) to give guidance on pre-clinical testing strategies and design for first-in-man clinical trials for new medicinal products [59], key pre-clinical studies, such as toxicity and pharmacokinetic and pharmacodynamic studies in non-human primates, will be needed to demonstrate that anti-CD151 mAbs can be safely used in clinical trials in humans. Despite this cautionary note, a rapidly growing set of experimental data concurs to reinforce the idea that CD151 is a promising innovative target in oncology. mAbs targeting CD151 offer novel therapeutic perspectives to act at the level of metastasis, (neo)angiogenesis and, as we have demonstrated, primary tumour cell growth.

References


Received 24 January 2011
doi:10.1042/BST0390553

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