Human monoclonal antibodies in cancer therapy: a review of recent developments

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1. ABSTRACT

   In the last decade, phage-display technology for the generation of monoclonal antibodies (mAbs) has improved significantly. Several novel human mAbs directed to a wide range of targets have been generated for the treatment of common malignancies. These targets include antigens associated with apoptosis, angiogenesis and solid tumors, as well as tumor growth-related antigens, insulin-like growth factor I receptor and hepatocyte growth factor. The safety, pharmacokinetics, and pharmacodynamics of several human mAbs have been evaluated in patients with advanced solid tumors. In conclusion, significant advances in the generation and application of human mAbs in cancer therapy have been made in the last decade.

2. INTRODUCTION

   Monoclonal antibodies (mAbs) are monospecific antibodies made by identical immune cells that are all clones of a unique parent cell. mAbs have monovalent affinity, in that they bind to the same epitope. The mAbs were discovered in the first half of the seventies by Milstein and Köhler, who selected only hybrid cells and clones with known specificity (1). Since then, it has become possible to generate unlimited amounts of antibodies with specificity against any cellular antigen. Ever since the discovery that mAbs could be generated in vitro, scientists have targeted the creation of ‘fully’ human mAbs to avoid some of the side effects of humanized and chimeric antibodies. Human mAbs offer more advantages because they are less antigenic and better tolerated; furthermore, they have a
longer circulation time in comparison with chimeric antibodies. Two successful approaches, phage display-generated antibodies (2) and genetically engineered mice (3), were identified to produce more human-like antibodies.

Antibody-based therapeutics has emerged as an important component in the treatment of an increasing number of human malignancies. Human mAbs have led to major advances in the treatment of common malignancies including melanoma (e.g., ipilimumab), chronic lymphocytic leukemia (e.g., ofatumumab) and non-Hodgkin lymphoma (e.g., tositumomab). These human mAbs were approved for use as anticancer agents during the last decade, reflecting the significant development of mAbs for cancer therapy in recent years. Here, we review the development of mAb therapy of cancer during the last decade, emphasizing the recent advances in the identification of targets and the generation of mAbs for the treatment of common malignancies such as melanoma, medullary thyroid cancer and glioblastoma.

3. NOVEL METHODS OF HUMAN MONOCLONAL ANTIBODY PRODUCTION DEVELOPED IN THE LAST DECADE

The display of library-derived antibody fragments on the surface of filamentous bacteriophages is a new technology introduced in the last decade of the 20th century for the identification of large repertoires of genes coding for the variable regions of the Ig chains in humans (2). In the last decade, the technology of phage display-generated antibodies has improved considerably. Huls and colleagues (4) developed a method that allows the rapid improvement of the affinity of phage-displayed antibody fragments by selection on intact eukaryotic cells. In this method, a single chain Fv fragment, specific for the tumor-associated Ep-Cam molecule, was mutagenized by shuffling of the immunoglobulin light chain variable region and DNA shuffling of both heavy and light chain variable regions. Higher-affinity mutants were selected from small phage display libraries by cell panning under stringent conditions. When converted to an intact fully human antibody, the mutagenized anti-tumor mAb displayed an affinity of 0.4 nM, a 15-fold improvement over the affinity of the original antibody. The performance of the higher-affinity antibody in complement-mediated tumor cell killing was also improved. Later, Fujiki and colleagues (6) succeeded in obtaining antigen-specific antibody genes by the phage-display method. Furthermore, by combining the variable-region genes and constant-region genes of human IgG, they obtained four independent human mAbs specific for tumor necrosis factor-alpha, demonstrating the effectiveness of this strategy for generating antigen-specific human mAbs using a peptide antigen (5).

The identification of rare tumor-reactive mAbs in large phage display mAb libraries requires affinity-selection methodologies. It is important but not always possible to develop a more general approach for discovering disease- or tissue-specific human mAbs that does not require intentional immunization. Wu and colleagues (6) constructed a phage-expressed antibody library from human colon tumor-infiltrating B lymphocytes, and they isolated and characterized tumor-reactive antigen binding fragments (Fabs) to develop a rapid approach for identifying human IgGs reactive with cell surface tumor antigens. They combined the advantages of generating Ab libraries from an enriched source with an assay strategy that permitted the screening of every member of the library. The relatively small size of the tumor-derived library allowed direct screening of soluble Fabs of every member of the library, permitting the characterization of multiple human mAbs that might not be discovered using alternative approaches, such as hybridoma technology or phage-display.

Since the development of phage-display technology, several other means of displaying antibodies have been proposed, such as display on ribosomes. Compared to phage display, ribosome display has a number of advantages as a methodology for the identification and selection of antibodies. First, ribosome display is not limited by the transformation step characteristic of other library construction methods, thus enabling the generation of a larger and more diverse library for selecting the highest affinity and specificity antibodies. Furthermore, the transcription, translation, and panning are performing in a cell-free system, which allows for the expression of toxic proteins and circumvents random mutations introduced by PCR (7). In addition to its application in the selection of ligand-binding proteins (8,9), enzymes (10), and other peptides (8,11), ribosome display has been used successfully for the selection of antibody fragments (12,13), including anti-Fas antibodies (14), Fab molecules (15) and single-chain variable fragment (scFv) antibodies (16,17). Ribosome display has also been reported to be effective for the selection of recombinant antibodies (18,19). Ribosome display using the reconstituted cell-free protein synthesis system can be applied for the epitope mapping of mAbs (20). A combination of error-prone PCR with ribosome display was used to screen each amino acid position within the humanized anti-RAGE mAb for its functional importance and its capacity to increase affinity (21). Ribosome display has been described as an effective method for the selection of humanized antibodies (22,23) and the generation of fully human antibody fragments in vitro (24). However, reports on the selection of human mAbs by ribosome display are scarce. The advantages of ribosome display suggest that this may be a promising technology for the selection of human mAbs for cancer therapy.

4. GENERATION OF NOVEL HUMAN MONOCLONAL ANTIBODIES

In parallel to the improvement of methodologies for the preparation of human mAbs, several novel human mAbs have been generated for the treatment of common malignancies. The monoclonal antibody 225.28S, which is specific against human high molecular weight-melanoma associated antigen (HMW-MAA), has been reported to suppress human melanoma tumor growth in SCID mice, as in vitro data suggested that this antigen plays a role in the spreading, migration and invasion of melanoma cells (25).
A neutralizing human mAb, 3G3, targeting the platelet-derived growth factor receptor alpha (PDGFR alpha), which is a type III receptor tyrosine kinase expressed in a variety of tumor types, was found to inhibit the growth of tumor xenografts (26). The 3G3 antibody could block ligand-induced cell mitogenesis, receptor autophosphorylation, and phosphorylation of the downstream signaling molecules Akt and mitogen-activated protein kinase. Thus, 3G3 may be useful for the treatment of tumors that express PDGFR alpha (26).

HoAKs-1, a novel human mAb produced successfully on tumor-infiltrating lymphocytes derived from lung cancer samples, was shown to inhibit cancer cell growth and induce morphological changes (27). HoAKs-1 showed specific and intense reactivity against the cell membrane of human lung adenocarcinoma HLC-1 cells and human pancreatic cancer PANC-1 cells, whereas the antibody did not show reactivity to human umbilical vein endothelial cells. In xenografts formed from epithelial cancer cell lines in nude mice, the antibody showed a broad spectrum of reactivity in 6 out of 14 xenografts. Moreover, in surgically resected clinical specimens, HoAKs-1 showed specific reactivity to cancerous lesions from patients with lung cancer but not to normal sites. Thus, HoAKs-1 shows potential as an anticancer agent with good specificity that does not trigger generalized immune reactions in humans (27).

Several novel human mAbs for cancer therapy have been generated recently that can be directed toward a wide range of targets including cell-surface proteins in solid tumors and circulating malignant cells, and antigens associated with apoptosis or the tumor stroma.

4.1 Targets in apoptosis

It has been known for many years that a massive cell loss takes place during carcinogenesis. Kerr and colleagues (28) were the first to suggest that apoptosis is the process responsible for that cell loss, and this idea was supported by subsequent studies showing that a large percentage of apoptotic cells are present in spontaneously regressing tumors and tumors exposed to cytotoxic agents. The growing knowledge on the relation between apoptosis and carcinogenesis has led to the development of human mAbs targeting apoptosis in cancer therapy. The human mAb PAM-1, which was isolated from a patient with stomach cancer, can inhibit cell growth and induce apoptosis in vitro and in vivo (29). The growth inhibitory effect of the PAM-1 antibody makes it a good therapeutic tool for all kinds of epithelial cancers and precursor lesions.

4.2 Targets in angiogenesis

Angiogenesis is required for invasive tumor growth and metastasis and constitutes an important process controlling cancer progression. It is therefore a promising target for antibody-mediated cancer therapy. The extracellular matrix (ECM) (EDA), which can be inserted in the fibronectin transcript by a mechanism of alternative splicing, has been shown to preferentially accumulate around new blood vessels in certain tumors. Human mAbs, namely F8, B7 and D5, have been generated that recognize the same epitope of EDA but differ in terms of their dissociation constant to the human antigen. Among these mAbs, F8 is a high-affinity human mAb specific to the alternatively spliced EDA domain of fibronectin that efficiently targets tumor neo-vasculature in vivo (30).

Angiopoietin 2 (Ang2), a ligand of the endothelial receptor tyrosine kinase Tie-2, is an important regulator of angiogenesis, blood vessel maturation and the integrity of the vascular endothelium. The correlation between the dynamic expression of Ang2 in tumors with regions of high angiogenic activity and a poor prognosis in many tumor types makes Ang2 an ideal drug target. A human anti-Ang2 mAb named MEDI3617 was generated that neutralizes Ang2 by preventing its binding to the Tie2 receptor in vitro, and inhibits angiogenesis and tumor growth in vivo (31). Treatment of mice with MEDI3617 resulted in the inhibition of angiogenesis in several mouse models including FGF2-induced angiogenesis in a basement extract plug model, and tumor and retinal angiogenesis. In xenograft tumor models, treatment with MEDI3617 resulted in a reduction in tumor angiogenesis and an increase in tumor hypoxia. The administration of MEDI3617 as a single agent to mice bearing human tumor xenografts significantly inhibited tumor growth by blocking angiogenesis in a broad spectrum of tumor types. Combination therapy with MEDI3617 and chemotherapy or bevacizumab resulted in a delay in tumor growth without loss of body weight. These data show that MEDI3617 is a robust antiangiogenic agent and support the clinical evaluation and biomarker development of MEDI3617 in cancer patients (31).

4.3 Insulin-like growth factor I receptor as a target

The insulin-like growth factor I receptor (IGF-IR) can stimulate cell proliferation and cell differentiation, induce changes in cell size, and protect cells from apoptosis. The overexpression of IGF-IR in tumor cells, often in concert with overexpression of insulin-like growth factor (IGF) ligands, leads to potentiation of signals and, as a result, enhanced cell proliferation and survival. A fully human mAb to the IGF-IR was generated that can block ligand-dependent signaling and inhibit human tumor growth in vivo (32). This fully human mAb (A12)-mediated blockade of ligand binding to the IGF-IR inhibited downstream signaling of the two major IGF pathways, mitogen-activated protein kinase and phosphatidylinositol 3'-kinase/Akt, in MCF7 human breast cancer cells. As a result, the mitogenic and proliferative potential of the IGF-IR ligands IGF-I and IGF-II were significantly reduced. A12 blocked ligand binding to an atypical IGF-IR in MCF7 cells. In addition, A12 induced IGF-IR internalization and degradation on specific binding to tumor cells, resulting in a significant reduction in cell surface receptor density. In xenograft tumor models in vivo, IGF-IR blockade by A12 led to significant growth inhibition of breast, renal, and pancreatic tumors. Histological analysis of tumor sections demonstrated a marked increase in apoptotic tumor cells in antibody-treated animals. These findings demonstrate that A12 possesses strong antitumor activity in vitro and in vivo and may therefore be an effective therapeutic candidate for the treatment of cancers that are dependent on IGF-IR.
signaling for growth and survival (32). Another human mAb targeting the IGF-IR was generated and named CP-751,871 (33). This antibody can block binding of IGF-I to its receptor and IGF-I-induced receptor autophosphorylation, and can down-regulate IGF-IR in vitro and in tumor xenografts. Combination therapy with these IGF-IR mAbs could be useful in cancer therapy to enhance the inhibition of tumor growth (33).

Using a phage display library, several high-affinity fully human mAbs with inhibitory activity against human IGF-IR were identified (34). The candidate therapeutic antibodies recognized several distinct epitopes and effectively blocked ligand-mediated receptor signal transduction and cellular proliferation in vitro. They also induced IGF-IR downregulation and catabolism following antibody-mediated endocytosis. These antibodies, namely IGF-IR antibodies 7A6, 9A2, and 12A1 inhibited (125I)-IGF-I binding, and IGF-IR antibodies 7A4, 8A1, and 9A2 inhibited (125I)-labeled IGF-II binding to NIH-3T3 cells expressing the human IGF-IR. These antibodies exhibited activity against human, primate, and rodent IGF-IRs, and dose-dependently inhibited the growth of established human tumors in nude mice (34). Human mAbs to the IGF-IR were shown to inhibit receptor activation and tumor growth in preclinical studies. Furthermore, the effectiveness of human mAbs to the IGF-IR was examined in a study of patients with advanced solid tumors. In a phase I study evaluating the pharmacokinetics, pharmacodynamics, safety, and tolerability of R1507, a fully human IgG1 type mAb against the human IGF-IR, R1507 was well tolerated by patients and it had antitumor activity in patients with solid neoplasms, in particular Ewing’s sarcoma (35).

4.4 Targets in hepatocyte growth factor-mediated signaling

The receptor for hepatocyte growth factor (HGF), c-Met, is a well-characterized receptor tyrosine kinase that mediates a variety of epithelial cell functions, and dysregulation of the HGF/c-Met pathway has been implicated in several human malignancies (36,37). The HGF/c-Met pathway mediates a plethora of normal cellular activities including proliferation, survival, migration, invasion, and branching morphogenesis (36,37), which are at the root of cancer cell dysregulation, tumorigenesis, and tumor metastasis. Therefore, inhibiting HGF-mediated signaling could be an effective therapeutic approach for treating patients with a broad spectrum of human tumors. Five different fully human mAbs were generated from single clones of five hybridomas, and shown to bind to and neutralize human HGF (38). These antibodies with subnanomolar affinities for HGF blocked binding of human HGF to c-Met and inhibited HGF-mediated c-Met phosphorylation, cell proliferation, survival, and invasion. More importantly, these antibodies inhibited HGF-dependent autocrine-driven tumor growth, and caused significant regression of established U-87 MG tumor xenografts (38). These results suggest that an antibody to an epitope in the beta-chain of HGF has potential as a novel therapeutic agent for treating patients with HGF-dependent tumors.

Later, another two human anti-Met mAbs (R13 and R28) with antitumor activity against colon cancer tumor models in vivo were discovered (39). These two mAbs can synergistically inhibit HGF binding to Met, elicit antibody-dependent cellular cytotoxicity, and abrogate HGF-induced activation of MET, AKT1, ERK1/2, and HGF-induced migration and proliferation. The inhibitory effect was mediated by “locking” the Met receptor in a state with R13, which then increased the avidity of R28 for the extracellular domain of Met, thus blocking HGF binding without activating the receptor (Figure 1). The combination of R13/28 significantly inhibited tumor growth in various colon tumor xenograft models and this effect was associated with the induction of hypoxia. Furthermore, global gene expression analysis showed that inhibition of the HGF/Met pathway significantly upregulated the tumor suppressors KLF6, CEACAM1, and BMP2, the negative regulator of phosphatidylinositol-3-OH kinase PIK3IP1, and significantly suppressed SCF and SERPINE2, both enhancers of proliferation and invasiveness. Moreover, in an experimental metastasis model, R13/28 increased survival by preventing the recurrence of otherwise lethal lung metastases (39).

4.5 Targets in solid tumors

Human mAbs for cancer therapy can be directed toward solid tumors. A phage display-derived human mAb (HNS76) with similar binding characteristics to the chimeric tumor necrosis therapy (TNT)-1 antibody, which is directed against necrotic regions of solid tumors, was recently generated (40). TNT is an approach to tumor targeting based on mAbs directed against common intracellular antigens such as nucleic acids, accessible only in necrotic areas of solid tumors. By binding to the necrotic core of tumors, these TNT mAbs can circumvent many of the limitations of MAbs directed against tumor cell surface antigens (40). In an in vivo study, the human mAb G11 to domain C of tenasin-C, which selectively targets solid tumors, was generated by antibody phage technology (41). Because the extra-domain C of tenasin-C is strongly expressed in the majority of lung cancers, and the antibody’s ability to preferentially localize at the tumor site was confirmed in an orthotopic rat glioma model, the G11 antibody could be used as a portable targeting moiety for the selective delivery of imaging and therapeutic agents to gliomas and lung tumors (41).

5. HUMAN MONOCLONAL ANTIBODIES AND CYTOTOXIC ACTIVITY

In cancer immunotherapy, effective therapeutic Abs are typically thought to require both cytostatic and cytotoxic abilities (42). This can be optimally achieved if the Ab activates potent cytotoxic immune effector functions such as Ab-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The specific cytotoxic activity of a novel human mAb (MT201) against primary ovarian tumor cells was evaluated (43). MT201 is a novel fully human Ep-CAM-specific IgG1 antibody that can effectively redirect tumor-resident effector cells against Ep-CAM-positive ovarian cancer cells and may therefore be a promising therapy for
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Figure 1. mAbs R13 and R28 synergistically inhibit HGF binding to Met. R13 locks the Met receptor, and then the avidity of R28 for the extracellular domain of Met is increased, leading to the blockade of HGF binding without activating the receptor. As a result, antibody-dependent cellular cytotoxicity is elicited, and the HGF-induced activation of MET, AKT1, ERK1/2, and HGF-induced migration and proliferation are all abrogated.

ovarian cancer (43). Daratumumab is a novel high-affinity therapeutic human mAb against a unique CD38 epitope whose cytotoxic mechanism of action was described recently. Daratumumab can effectively kill multiple myeloma tumor cells in a tumor-preserving bone marrow microenvironment. In vivo, daratumumab was highly active and interrupted xenograft tumor growth at low doses. Collectively, these results show the versatility of daratumumab to effectively kill CD38-expressing tumor cells, including multiple myeloma cells, via diverse cytotoxic mechanisms (44).

6. CLINICAL APPLICATION OF MONOCLONAL ANTIBODIES IN THE LAST DECADE

Several novel human mAbs have been demonstrated to be effective and safe in patients with malignancies. A fully human mAb to anti-alpha(v) integrin (CNTO 95) was shown to inhibit angiogenesis and tumor growth in a phase I trial that included 24 patients with advanced refractory solid tumors (45). This study showed the penetration of the tumor by CNTO 95 and its localization to tumor cells. Exposure to the antibody seemed to increase in a greater-than-dose-proportional manner; CNTO 95 had a dose-dependent mean half-life of 0.26 to 6.7 days, and was generally well tolerated (45).

The safety, pharmacokinetics, and pharmacodynamics of several novel human mAbs have been assessed in patients with advanced solid tumors. Mapatumumab (HGS-ETR1, TRM-1), a fully human agonist mAb directed to the tumor necrosis factor-related apoptosis-inducing ligand receptor-1 (TRAIL-R1) was assessed in a phase I pharmacokinetic and biologic correlative study that included 49 patients with advanced solid malignancies, and the results showed that mapatumumab can be administered safely and feasibly at 10 mg/kg IV every 14 days. The absence of severe toxicities and the attainment of plasma mapatumumab concentrations that are active in preclinical models warrant further disease-directed studies of this agent alone and in combination with chemotherapy in a broad array of tumors (46). The pharmacokinetics and safety of subcutaneously administered weekly ING-1, a high-affinity, human engineered mAb targeting epithelial cell adhesion molecule were evaluated in 14 patients with advanced refractory
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solid tumors (47), and the results showed that ING-1 administered subcutaneously at 0.6 mg/kg/week was well tolerated, suggesting that further studies at this dose are warranted to confirm its safety and effectiveness. The risk of pancreatitis associated with this antibody and its marginal anti-tumor effect may preclude further monotherapy studies; however, combination studies with chemotherapy are warranted (47). Gordon and colleagues (48) reported the safety, pharmacokinetics, and pharmacodynamics of AMG 102, a fully human hepatocyte growth factor/scatter factor (HGF/SF)-neutralizing mAb in 40 patients with advanced solid tumors, and further found that AMG 102 is safe and well tolerated, has a favorable pharmacokinetic profile, and will be further investigated as a monotherapy and in combination with other agents (48).

The safety and clinical activity of L19-TNF, a tumor-targeting immunocytokine composed of the human L19 antibody binding to extra domain B (ED-B) of fibronectin of newly formed blood vessels and TNF, plus melphalan-containing isolated limb perfusion (ILP) in extremity melanoma patients were evaluated in a recent study (49). The results showed that ILP with L19-TNF had a favorable safety and a promising activity profile at a dose of 650 µg of L19-TNF, supporting the exploration of higher L19-TNF doses and a Phase II trial comparing L19-TNF ILP with standard melphalan-containing ILP (49).

7. CONCLUSIONS

In the last decade, advances in the production of human mAbs have resulted in the generation and characterization of several novel human mAbs for application in cancer therapy. In the future, the identification of novel targets will likely trigger the development of additional cancer-specific human mAbs, and further studies should improve the effectiveness of existing mAbs for the treatment of cancer.

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9. REFERENCES


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**Abbreviations:** mAbs: Monoclonal antibodies; HMW-MAA: human high molecular weight-melanoma associated antigen; PDGFR alpha: platelet-derived growth factor receptor alpha; EDA: extra-domain A; Ang2: Angiopoietin 2; IGF-IR: insulin-like growth factor I receptor; HGF: hepatocyte growth factor; TNT: tumor necrosis therapy; ADCC: Ab-dependent cellular cytotoxicity; CDC: complement-dependent cytotoxicity; TRAIL-R1: tumor necrosis factor-related apoptosis-inducing ligand receptor-1; HGF/SF: hepatocyte growth factor/scatter factor; ED-B: extra domain B; ILP: isolated limb perfusion

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