Review Article

Monoclonal antibodies as effective therapeutic agents for solid tumors

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(Received April 16, 2004/Revised June 1, 2004/2nd Revised June 8, 2004/3rd Revised June 9, 2004/Accepted June 10, 2004)

Monoclonal antibodies (mAbs) against growth factors or their receptors have been revealed to be effective therapeutic agents for solid tumors. Trastuzumab (humanized anti-HER2 mAb) is the first mAb approved for the treatment of a solid tumor, metastatic breast cancer. Large-scale phase III clinical trials are now ongoing to further evaluate the additive effects on chemotherapy and the efficacy as a maintenance monotherapy. Another anti-HER2 mAb CH401 that we developed also seems to have good potential. This chimeric mAb completely suppressed the growth of established human tumor xenografts in SCID mice after a single injection. Furthermore, CH401 characteristically showed much stronger induction of apoptosis in HER2-overexpressing gastric cancer cells compared to trastuzumab. Additional targets now being intensively evaluated are epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF). Both cetuximab (chimeric anti-EGFR mAb) and bevacizumab (humanized anti-VEGF mAb) have recently been shown to be of clinical value for metastatic colorectal cancer. Anti-idiotype mAbs are unique as active immunotherapeutic agents, and survival benefits have been observed in clinical trials for solid tumors. (Cancer Sci 2004; 95: 621–625)

The development of a laboratory technique for making monoclonal antibodies (mAbs) by Milstein and Köhler in 1975[1)] allowed the massive production of mAbs against cancer cells. Consequently, it was expected that highly specific and effective antibody therapy would be developed. However, the results obtained from several clinical trials in the early 1980s revealed limited clinical responses and adverse effects mainly due to the xenogenicity of the mAbs. Through numerous subsequent preclinical studies on a variety of mAbs, the efficacy of shutting off receptor-mediated signaling as a means of blocking cell growth and viability was noted. Remarkable objective responses were observed during clinical trials using rituximab (chimeric anti-CD20 mAb)[2)] or trastuzumab (humanized anti-HER2/neu/ErbB-2 mAb),[3)] resulting in a second wave of mAb therapy. Various mAbs against human epidermal growth factor receptor 2 (HER2),[3–7)] epidermal growth factor receptor (EGFR),[8–13)] vascular endothelial growth factor (VEGF),[14, 15)] and VEGF receptor (VEGFR)[16)] were then subjected to clinical trials. In addition to this mAb group, anti-idiotype mAbs are worthy of note, since they are uniquely used for active immunotherapy. This article reviews some of the recent remarkable findings concerning cancer therapy with these mAbs, especially against solid tumors.

Anti-HER2 mAbs

Trastuzumab (Herceptin) is a humanized mAb, which was approved by the United States Food and Drug Administration in 1998 for the treatment of advanced breast cancer. This was the first approval of a mAb for use in solid tumor therapy. Three years later, it was approved in Japan. The approved use of trastuzumab in HER2-positive metastatic diseases includes as a first-line treatment in combination with paclitaxel and as a monotherapy in patients who have received one or more chemotherapeutic regimens. HER2 overexpression is observed in 15–30% of breast cancers.[17, 18)] There are currently four major phase III multicenter trials that in total will randomize approximately 12,000 patients to chemotherapeutic regimens with or without trastszumab.[19)] HER2 expression is examined using validated fluorescence *in situ* hybridization or immunohistochemistry assays. The duration of trastuzumab maintenance is 1 or 2 years after chemotherapy with or without trastuzumab. In Japan, Sawaki *et al*.[20)] reported the clinical response of 27 metastatic breast cancer patients to trastuzumab as a single agent. Complete response, partial response, no change, and progressive disease were observed in 3, 3, 3 and 17 patients, respectively, with one case being not evaluated, and the therapy was well tolerated.

The most serious, but unexpected, toxicity observed during the pivotal trials was cardiac dysfunctions, including clinically manageable left ventricular systolic dysfunction, and occasionally advanced congestive heart failure in a small percentage of patients.[21)] The incidence and severity of cardiac dysfunction was greatest in patients receiving concomitant trastsuzumab and anthracycline plus cyclophosphamide.[22)] Although the pathophysiological mechanisms of cardiac dysfunction associated with trastuzumab are not clearly understood, Crone *et al*. demonstrated that mice with a ventricular-restricted deletion of the *HER2* gene developed dilated cardiomyopathy,[23)] indicating that HER2 signaling in the cardiomyocytes is essential for the prevention of this disorder.

HER2 expression is found in solid tumors other than breast cancer. Some objective responses have been observed during clinical trials for colorectal and non-small cell lung cancers.[24, 25)] Pancreas,[26)] bile duct[27)] and stomach[4, 28)] cancers that express HER2 with a relatively high frequency are expected to be new targets of anti-HER2 mAb therapy.

We developed several mAbs against HER2[4)] and prepared a mouse-human chimeric mAb CH401 using the mAb, E401, which showed the most potent *in vitro* cytolytic activity, to reduce immunogenicity and enhance effector functions.[29)] Indeed, CH401 was much more efficient than the mouse mAb E401 in antibody-dependent cell-mediated cytotoxicity assay (ADCC)

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Abbreviations: mAb, monoclonal antibody; HER2, human epidermal growth factor receptor 2; ADCC, antibody-dependent cell-mediated cytotoxicity; SCID, severe combined immunodeficiency; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; 5-FU, 5-fluorouracil; LV, leucovorin.

with human effector cells.[29] The most striking finding was its *in vivo* anti-tumor effect. The efficacy of CH401 therapy was tested on the growth of gastric cancer JRST cells using a mouse model with tumor xenografts. Antibodies were administered to mice only once, when the tumor of JRST cells was established. As shown in Fig. 1, they produced rapidly growing tumors with a short latency treated with an irrelevant antibody, chimeric anti-CD54 mAb,[30] whereas they did not form tumors at all in 95% of mice treated with 200 μg of CH401 (to be published). This therapeutic effect was not restricted to JRST cells. The *in vitro* cytolytic activity of CH401 was demonstrated in all the HER2-expressing human cultured cell lines tested (Fig. 2).[31] In these experiments, cells were incubated with CH401 for 48 h and viable cells were simply determined by trypan blue staining, suggesting that the *in vivo* anti-tumor effect was at least partly due to CH401 alone. It is also noteworthy that more than 50% of cells were killed irrespective of the HER2 expression level.[31]

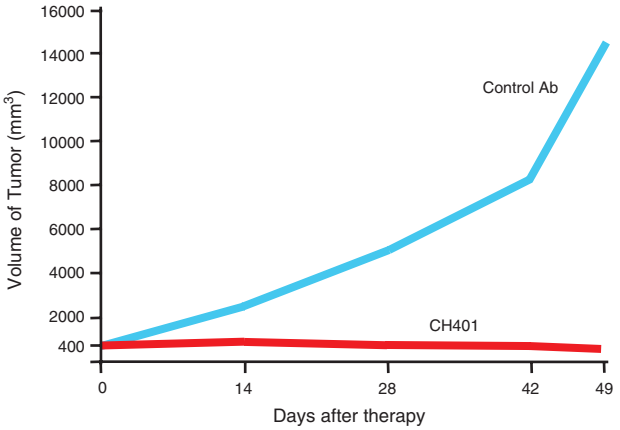

Fig. 1. Anti-tumor activities of mAb CH401 against human gastric cancer JRST cells in SCID mice. Chimeric anti-CD54 mAb was used as a control antibody. 1×10^6 human gastric cancer JRST cells were s.c. injected into the flanks of SCID mice. Antibodies were i.p. administered to mice only once when the tumor volume reached 400 mm^3. The size of the s.c. tumor in each mouse was measured once a week.

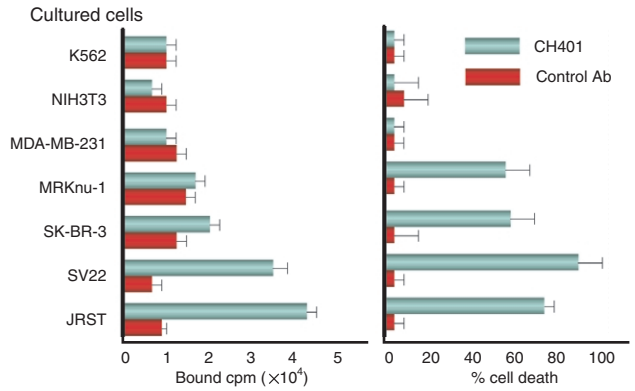

Fig. 2. Expression level of HER2 (*left*) and sensitivity to cytotoxic activity of mAb CH401 (*right*) in cultured tumor cells. Chimeric anti-CD54 mAb was used as a control antibody. *Left*, Cultured cells were incubated for 2 h at 4°C with ^{125}I-labeled mAbs, then washed, and bound radioactivity of cells was counted in a γ-counter. *Right*, Cells were incubated with mAbs. Aliquots of cells were removed at 48 h and viable cells were determined by trypan blue staining. The percentage cell death was calculated as (the dead cell number/non-treated cell number per milliliter)×100. All the HER2-positive cells were susceptible to cytolytic activity of mAb CH401, while the HER2-negative cells were not.

We then found that the cytolytic action of CH401 was based on programmed cell death. Morphological changes and DNA fragmentation were recognized at least 12 h after treatment of

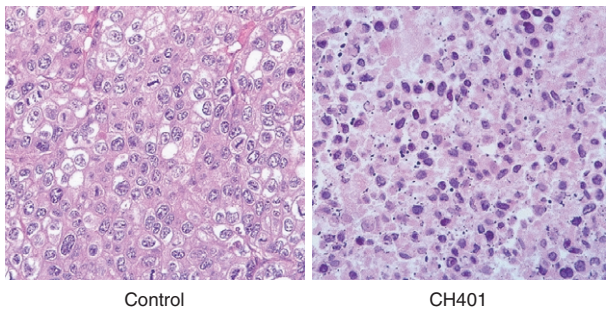

Fig. 3. Apoptosis in tumor tissues of gastric cancer JRST cells at 7 days after treatment with mAb CH401 (H-E staining). Control tissues were obtained from mice treated with an irrelevant mAb. Tumor formation in SCID mice and antibody administration were as described in Fig. 1.

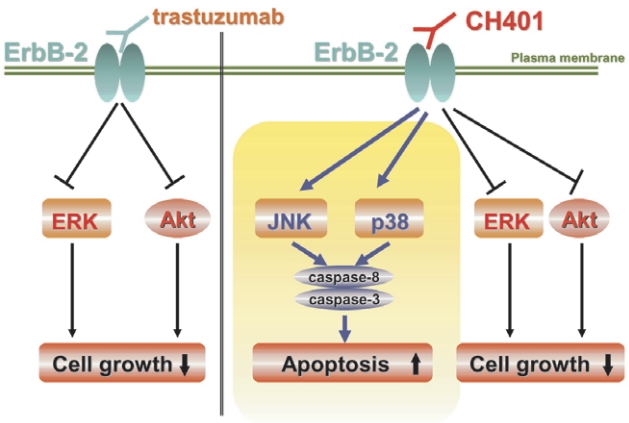

Fig. 4. Schematic presentation of the possible mechanism of growth-suppressing activity of CH401 or trastuzumab. CH401 inhibits ERK and Akt, while it activates p38 MAPK and JNK, resulting in the suppression of cell growth and the induction of apoptosis. Only the inhibition of Akt activation has been shown for trastuzumab.

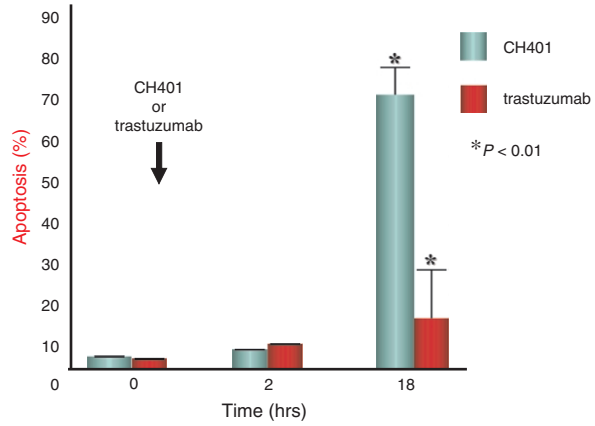

Fig. 5. Comparison of inducibility of apoptosis in SV22 cells between CH401 and trastuzumab. SV22 cells (HER2 transfectants of NIH3T3 cells) were incubated with antibodies (50 μg/ml) for up to 18 h at 37°C and apoptosis (%) was measured with an apo-percentage kit. CH401 induced apoptosis in more than 70% of SV22 cells at 18 h, whereas trastuzumab did so in only 10–20% of them.

cultured cells with CH401.[31] Apoptotic changes of tumor cells were also observed *in vivo* after administration of CH401 (Fig. 3) (unpublished data). Although the entire picture of the molecular mechanism for induction of apoptosis with CH401 remains to be established, we have found that CH401 suppresses the cell growth by inhibiting the activation of ERK and Akt, while it induces apoptosis through the activation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK), as shown in Fig. 4 (to be published). It was reported that trastuzumab inhibited Akt activation, but it remains unclear if it affects the activity of p38 MAPK and/or JNK. We compared the apoptosis induction between trastuzumab and CH401 after incubating SV22 cells (HER2-transfectants of NIH3T3) with each mAb. As shown in Fig. 5, the percentage of apoptotic cells was more than 70% in CH401 treatment, whereas it was only 10–20% in the case of trastuzumab (unpublished data). A previous study also showed that the extent of apoptosis (Apo-BrdU analysis) induced by trastuzumab alone in breast cancer SKBR-3 cells was 10.5%.[32] When trastuzumab was incubated with breast cancer BT474 cells together with pertuzumab, another anti-HER2 mAb, the percentage of cells staining positive for annexin V-PE and/or 7-AAD was 20–25%.[33] These data suggest that apoptosis induction by CH401 may be remarkably high among anti-HER2 mAbs so far developed. A comparison of the *in vivo* anti-tumor effect between CH401 and trastuzumab is now under way in our laboratory. It was recently shown that trastuzumab-mediated ADCC was impaired in peripheral blood mononuclear cells from patients with advanced gastric cancer, which correlated with the down-regulation of CD16zeta expression in NK cells.[34] Although it will be necessary to see whether this phenomenon generally occurs in advanced cancer patients, CH401 might be more potent under these conditions than trastuzumab.

Anti-EGFR or VEGF mAbs

EGFR and VEGF/VEGFR are additional possible targets of mAb therapy. EGFR is expressed at a high frequency in a variety of tumors.[35] Clinical trials have therefore been performed in a number of cancers including head and neck, breast, lung, colorectal, pancreatic, kidney, bladder, ovarian and brain cancers. Although there are a number of mAbs being subjected to clinical trials, a murine-human chimeric mAb, cetuximab (C225), has been extensively evaluated and is the subject of most of the currently available clinical data.[8] In a recent clinical trial for colorectal cancer,[36] 329 patients with irinotecan-refractory metastatic colorectal cancer were randomized into two trial arms (arm A, cetuximab plus irinotecan; arm B, cetuximab alone). The median times of progression of arms A and B were 4.1 and 1.5 months, respectively, indicating a significant additional effect of cetuximab. The adverse characteristic of anti-EGFR-targeted therapy is an acne-like skin rash, which was observed in 77% of 813 patients treated in 21 trials.[37] Very recently, cetuximab has been approved in the United States and Europe for the treatment of irinotecan-refractory metastatic colorectal cancer.

Anti-angiogenesis therapy is unique in targeting tumor vasculature, but not tumor cells themselves, and therefore it is broadly applicable for most solid tumors.[38] Although numerous growth factors are involved in angiogenesis, VEGF is hypothesized to play a pivotal role in tumor angiogenesis.[14] Bevacizumab is a humanized anti-VEGF mAb, which has been most extensively investigated in a variety of tumors, including non-small cell lung, breast, prostate, renal and colorectal cancers.[14] The latest notable finding was a result of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil (5-FU), leucovorin (LV)) as the first-line therapy of 925 patients with metastatic colorectal cancer.[39] The median survival times of the IFL/placebo and IFL/bevacizumab groups were 15.6 and 20.3 months, respectively. Serious adverse effects such as hemorrhaging and thrombosis were reported in a clinical trial of bevacizumab with or without 5-FU/LV for metastatic colorectal cancer. Gastrointestinal bleeding, epistaxis and thrombotic events were seen in 10.3, ~50 and 19.1% of the patients receiving bevacizumab alone, respectively.[14]

Anti-idiotype mAbs

According to the idiotype network theory,[40] immunization with a given antigen will produce antibodies against this antigen termed Ab1. The Ab1 might generate a series of anti-idiotype antibodies against Ab1 termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structures of external antigens. These particular anti-idiotypes (Ab2β) can induce specific immune responses similar to those induced by the nominal antigens. Ab2β has the internal image of the antigen recognized by Ab1. We previously revealed that an anti-idiotypic mAb (Ab2β) against an anti-carcinoembryonic antigen (CEA) mAb MA208 can induce an antibody response specific for CEA,[41] and that the CDR2 in V_H and CDR3 in V_L of the Ab2β M7-625 have amino acid sequences similar to the domain III of CEA.[42] To examine whether Ab2β can induce Ab1-like Ab3, we then prepared anti-anti-idiotype mAbs using an Ab2β M315 against anti-synthetic CEA peptide mAb P1-356. A resultant mAb, 11B2, reacted directly with CEA and competed with mAb P1-356, suggesting that the mAb is Ab1-like Ab3.[43] Furthermore, the *in vivo* administration of an Ab2β D704, which was raised against a mAb M2590 recognizing a sialic acid residue of GM3 ganglioside, could induce the activity of anti-anti-idiotype antibodies (Ab3) that specifically reacted with GM3.[44]

Clinical trials with Ab2βs to mAbs that recognize tumor-associated antigens have been performed in solid tumors.[45–47] Although no apparent objective responses were seen in colorectal and ovarian cancer patients,[45, 46] some survival benefits were observed in both studies. In a study of advanced melanoma patients with an Ab2β that mimics GD2,[47] one patient had a complete response and 12 patients were stable from 14 to 37 months. A favorable effect of anti-idiotype antibody response on patients' survival was observed in several clinical trials with mAbs against solid tumors,[48–50] and this may support the validity of anti-idiotype mAb therapy.

Conclusions

The treatment of solid tumors with mAbs, especially against growth factors or their receptors has changed from an ineffective therapeutic modality into an effective one. A wide variety of those mAbs is now being developed and evaluated in preclinical and clinical settings. Since they target self/tumor antigens, it is not unexpected that adverse effects such as cardiac dysfunction of trastuzumab and thrombotic events in the case of bevacizumab have been found in clinical studies. Careful attention will have to be paid to adverse effects in mAb therapy, as is the case in conventional anti-cancer therapies. In contrast, the clinical effect of anti-idiotype mAbs on solid tumors except for malignant melanoma seems to be still modest. Altering the amino acid sequence of the internal image, as has been attempted in class I peptides, may be a possible strategy for enhancing the immunogenicity of those mAbs.

This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (C) (#12217097) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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