



HOKKAIDO UNIVERSITY

Title	Molecular cloning and characterization of antigens recognized by monoclonal antibodies targeting tumor endothelial cells
Author(s)	TANIGUCHI, Kenji
Citation	Japanese Journal of Veterinary Research, 50(2-3), 142-143
Issue Date	2002-11-29
Doc URL	https://hdl.handle.net/2115/2959
Type	departmental bulletin paper
File Information	KJ00002400484.pdf



neither increased the magnitude of pressor response to colonic distension in anesthetized rats nor decreased the visceromotor threshold to colorectal distension in conscious rats. These results suggest that the selective 5-HT₃ receptor agonist facilitates defecation without increasing visceral pain in rats.

In conclusion, it is indicated that 5-HT₃ receptors play roles in the induction of GMC, in the facilitation of defecation and in the in-

creasing of colonic secretion of water and electrolytes. Moreover, it is indicated that YM-31636 is a 5-HT₃ receptor agonist that selectively stimulates colonic motility, and thus could be promising as an agent against constipation, because it facilitates defecation or improves constipation without inducing emetic episodes, diarrhea and visceral pain. It is also suggested that the different types of 5-HT₃ receptor exist in the same species.

Original papers of this thesis appeared in *Eur. J. Pharmacol.*, 320 : 187-192 (1997), *Life Sci.*, 66 : PL 331-338 (2000), *Eur. J. Pharmacol.*, 409 : 195-201 (2000), *Eur. J. Pharmacol.*, 424 : 151-157 (2001) and *Eur. J. Pharmacol.*, 431 : 35-41 (2001)

Molecular cloning and characterization of antigens recognized by monoclonal antibodies targeting tumor endothelial cells

Kenji Taniguchi

*Pharmaceutical Research Laboratory II
Chugai Pharmaceutical Co., Ltd.
Gotemba 412-8513, Japan*

Solid tumors get nutrients and oxygen from the host blood supply and form new capillary vessels when the size exceeds 2 mm diameter. Chemotherapy shows cytotoxic effects to both tumor cells and highly proliferative normal tissues like bone marrow and intestine. Chemotherapy has to meet "total cell killing" of tumor cells to cure cancer patients. It is, however, very difficult for chemotherapeutic agents to reach all tumor cells in solid tumors, because tumor vessels do not run uniformly in solid tumors. Due to its low specificity and accessibility to tumor cells, chemotherapy can, thus far, hardly cure the patients of solid tumors. In contrast to its poor curability of solid tumors, the usefulness of targeting tumor vessels has been highlighted. Systemically administered agents targeting tumor vessels may block blood supply and inhibit

proliferation of surrounding tumor cells effectively.

Monoclonal antibodies (MAbs) had been generated against cultured tumor endothelial cells (TEC) separated from rat KMT-17 solid tumors and antigens of 40 kD and 80 kD were recognized by a series of the MAbs. Also, TES-23 MAb had shown anti-tumor effects on KMT-17 solid tumors and stained tumor vessels of human cancer tissues.

In the present study, molecular cloning and characterization of 40 kD and 80 kD antigens were conducted and the cross-reactivity of TES-23 MAb to human antigen was examined based on the possibility of its clinical use. Firstly, the 80 kD antigen, which was also expressed on ROS-17/2.8-5 rat osteosarcoma cells, was identified as rat hematopoietic CD44 (CD44 H) by panning screening of cDNA li-

brary of ROS-17/2.8-5 cells with TES-27 MAb. Then, the 40 kD and 80 kD antigens on rat TEC were identified as rat OTS-8 and CD44 H by screening of TEC cDNA library with TES-17 MAb and rat CD44 DNA probe, respectively. Immuno-histochemistry of KMT-17 solid tumors revealed that rat OTS-8 and CD44 were expressed on sprouting TEC. In addition, TES-23 MAb stained TEC of tubular vessels as well as sprouting TEC. PCR and northern blot analysis showed that CD44 mRNA with a splice in exon 6 was present in rat TEC at low levels.

Secondly, the cross-reactivity of TES-23 MAb to human antigen was determined. Immuno-precipitation of HT-1080 human fibrosarcoma revealed that TES-23 MAb cross-reacted human CD44. Flow cytometry revealed that TES-23 MAb reacted to HT-1080 cells almost comparably to anti-human CD44

MAb and moderately reacted to human umbilical vein endothelial cells (HUVEC), however, it hardly reacted to human peripheral blood mononuclear cells (hPBMC). The dependence of such differential reactivity on the activated form of human CD44 was examined by analyzing the binding of soluble hyaluroate and TES-23 MAb reactivity to human cells by flow cytometry. The binding of soluble hyaluronate to HT-1080 cells and HUVEC was clearly noted, but not to hPBMC. In addition, stimulation with phorbol 12-myristate 13-acetate induced soluble hyaluronate binding of MOLT-4 human T lymphoma cells and relatively increased the reactivity of TES-23 MAb. Our results suggest that TES-23 MAb may be useful for the treatment of human solid tumors based on less likelihood of major side effects to hPBMC in systemic administration of TES-23 MAb.

The original paper of this thesis appeared in *Int. J. Cancer*, 86 : 799-805 (2000) and *Biochem. Biophys. Res. Commun.*, 269 : 671-675 (2000).

Studies for the control of pandemic influenza :
Surveillance of animal influenza and the development of mucosal vaccines

Ai Ninomiya

*Laboratory of Microbiology, Department of Disease Control
Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*

Pig serum samples collected in southeastern China were examined for antibodies to influenza A viruses. Since the hemagglutination-inhibition (HI) test does not accurately detect antibodies to the hemagglutinins (HA) of "avian" influenza viruses, the author utilized the neutralization test to detect subtype-specific antibodies to the HA of avian viruses in pig sera.

Neutralizing antibodies to H1, H3, H4,

and H5 influenza viruses were detected in the serum samples collected in 1977-1982 and 1998, suggesting that pigs in China have been sporadically infected with avian H4 and H5 viruses in addition to swine and human H1 and H3 viruses. Antibodies to H9 virus, on the other hand, were found only in the sera collected in 1998, not in those collected in 1977-1982, correlating with the recent spread in poultry and subsequent isolation of H9N2 vi-