Clinical evidences of GM3 (NeuGc) ganglioside expression in human breast cancer using the 14F7 monoclonal antibody labelled with $^{99m}$Tc

Juan P. Oliva, Zodilina Valdéz, Angel Casacó, Gilmara Pimentel, Joaquín González, Irene Álvarez, Martha Osorio, Milagros Velazco, Mariela Figueroa, Rosa Ortiz, Xiomara Escobar, Maiby Orozco, Julia Cruz, Sonia Franco, Mirtha Díaz, Lourdes Roque, Adriana Carr, Ana M. Vázquez, Cristina Mateos, María C. Rubio, Rolando Pérez, and Luis E. Fernández

1Department of Nuclear Medicine, National Institute of Oncology and Radiobiology, Plaza, Havana, Cuba; 2Center of Molecular Immunology, Playa, Havana, Cuba; 3Obstetric and Gynecological Department, Luis Díaz Soto Hospital, Habana del Este, Havana, Cuba

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Abstract

The relevance of certain gangliosides in tumour growth and metastatic dissemination has been well documented, reasons for considering these molecules as potential targets for cancer immunotherapy and diagnosis. GM3(NeuGc) ganglioside is particularly interesting due to its restrictive expression in normal human tissues according to immunohistochemical studies, using either polyclonal or monoclonal antibodies. But both immunohistochemical and biochemical methods have strongly suggested its over-expression in human breast tumours. Nevertheless, the lack of a direct evidence of this antigenic display in human breast cancer has kept the subject controversial. For the first time, we described herein the “in vivo” detection of GM3(NeuGc) ganglioside in human breast primary tumours using a radioimmunoscintigraphic technique with 14F7, a highly specific anti-GM3(NeuGc) ganglioside monoclonal antibody, labelled with $^{99m}$Tc. In an open, prospective Phase I/II clinical trial, including women diagnosed in stage II breast cancer, the 14F7 monoclonal antibody accumulation in tumours at doses of 0.3 ($n=5$), 1 ($n=5$) and 3 mg ($n=4$) was evaluated. Noteworthy, the immunoscintigraphic study showed antibody accumulation in 100% of patients’ tumours for the 1 mg dose group. In turn, the radioimmunoconjugate injected at doses of 0.3 mg or 3 mg of the antibody, was uptaken by 60 and 33.3% of breast tumours, respectively. “In vivo” immune recognition of GM3(NeuGc) in breast tumours reinforces the value of this peculiar target for cancer immunotherapy.

Introduction

Gangliosides, sialic acid-containing glycosphingolipids, are expressed on the cell surface of vertebrates; they have engendered great interest for more than 20 years as potential targets for cancer immunotherapy and diagnosis due to their relevance for tumour growth and metastasis [1–3].

All tumours exhibit aberrant ganglioside expression. This includes the over-expression of gangliosides present in normal cells, which appear to be common among various tumours, and the expression of gangliosides not found in normal adult tissues [4].

$\alpha$-acetylated (NeuAc) and $\beta$-glycolyl (NeuGc) neuraminic acids are the most common types of sialic acids found in vertebrates [5]. In general, the NeuGc residue is not expressed in human and chicken normal tissues, but it is widely present in other vertebrates [6,7]. In contrast, it has been reported that anti-NeuGc-containing ganglioside antibodies recognize some human tumours and cancerous cell lines [8,9]. These studies have been carried out using polyclonal or monoclonal antibodies from chicken [10], human [11], or murine origin [12], but in all these tumours minimal levels of NeuGc were reported. On the other hand, we found recently increased levels of GM3(NeuGc) ganglioside in human breast tumors [13], a finding that certainly makes this molecule an attractive target for cancer therapy.

The 14F7 murine monoclonal antibody (MAb) is an IgG1 that was generated by immunizing Balb/c mice with GM3(NeuGc) ganglioside hydrophobically conjugated with human very-low-density lipoproteins and in the presence of Freund’s adjuvant. 14F7 MAb binds specifically to GM3(NeuGc) ganglioside, whereas neither any other NeuGc or NeuAc gangliosides, nor a sulphated glycolipids, are recognized as assessed by...
enzyme-linked immunosorbent assay or immunostaining on thin layer chromatograms. Immunohistochemical studies in fresh tumour tissues showed that 14F7 MAb strongly recognized the antigen displayed in human breast and melanoma tumours [14].

The 14F7 MAb agglutinates horse erythrocyte GM3(NeuGc)-positive cells and it is cytotoxic against GM3(NeuGc)-positive murine myeloma cells “in vitro” and “in vivo”. Curiously, this MAb directly kills target cells without the participation of complement. In addition, passive treatment with 14F7 MAb had a strong anti-tumour activity in P3 X63 Ag8 653 myeloma bearing mice, increasing survival similarly to the standard chemotherapy treatment [15].

It has been previously demonstrated by biochemical [13] and immunohistochemical [14] methods that GM3(NeuGc) ganglioside is expressed “in vitro” in human breast tumours. Herein we describe, for the first time, “in vivo” evidences of the presence of GM3(NeuGc) ganglioside in human breast primary tumors using a radioimmunoscintigraphic technique with the 14F7 MAb labelled with 99mTc.

Materials and methods

Study design

We performed an open, prospective and dose escalating Phase I/II clinical trial to evaluate the toxicity, and the immunoscintigraphic positive grade of the 14F7 MAb at doses of 0.3, 1 and 3 mg labeled with 30–40 mCi of 99mTc, in women with a clinical diagnosis of stage II breast cancer.

Patients

Fourteen women aged between 18 and 80 years with stage II breast cancer and with clinical, ultrasound, radiological and positive cytological diagnosis of breast malignant neoplasia were selected from the National Institute of Oncology and Radiobiology (INOR), Havana, Cuba. The diagnostic confirmation was performed by the histopathological study of tumour samples obtained during the surgical act.

Patients included in the study were without previous oncospecific treatment. Written informed consent was obtained from all patients.

Patients with any of the following characteristics were excluded: hemoglobin value less than 10 g/l; leucocytes count less than 4×109/l; platelet count less than 100×109/l; creatinine values higher than 132 mmol/l; serum transaminases and alkaline phosphatase without the normal reference values; patients with antecedents of chronic diseases such as asthma, ischemic cardiopathy, diabetes mellitus, hepatitis, uncompensated arterial hypertension, and pregnant or breastfeeding women.

Patients were divided into 3 groups. Group I received 0.3 mg of 14F7 (n = 5); group II received 1 mg (n = 5); and group III received 3 mg of 14F7 (n = 4) intravenously, always labelled with 30–40 mCi of 99mTc.

The dose scaling steepes were carried out only if in the previous group there was no severe or very severe toxicity according to the Common Toxicity Criteria of the National Cancer Institute (NCI-CTC).

The study was approved by the INOR’s Ethics Committee and the National Regulatory Authority of Cuba (CECMED).

Monoclonal antibody

14F7 is an IgG1 highly specific anti-GM3(NeuGc) MAb generated by immunization of mice with a vaccine formulation containing GM3(NeuGc) hydrophobically conjugated with human very low density lipoproteins [14]. Vials containing 1 ml of sterile and pyrogenic neutral solution with an antibody concentration of 5 mg/ml were supplied by the Center of Molecular Immunology, Havana, Cuba.

Antibody labelling

MAb was directly labelled according to the Schwarz’s method [16]. Briefly, intact antibody was reduced with 2000 molar excess of 2-mercaptoethanol (Sigma, St Louis, Missouri, USA) for 30 min at room temperature. Reduced antibody was purified from the excess of 2-mercaptoethanol using a Sephadex G-25 column (PD-10 gel filtration column, Pharmacia Biotech, Uppsala, Sweden). Protein concentration was determined measuring the optical density at 280 nm on an UV/visible spectrophotometer (Ultrospec III, Pharmacia).

A MDP bone-scanning kit (Medronato-Sn, CENTIS, Havana, Cuba) was reconstituted with 5 ml of 0.9% saline solution purged with nitrogen. Then, to aliquots containing 0.3, 1 or 3 mg of reduced 14F7 MAb, then 20, 50 or 120 μl of the medronate solution were, respectively added (according to group I, II, or III of patients), followed by 30–40 mCi of pertechnetate from a sterile Mo-99/Tc-99 m generator (GBTec, CENTIS, Havana, Cuba) eluted within the previous 24 h.

All radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. All glassware, plastics and solutions used were sterile and pyrogen free.

Quality control of Tc-99m labeled antibody

The amount of free pertechnetate and Tc-99m MDP were determined by ascending paper chromatography on Whatman 3 MM 0.5×8.0 cm strips using 0.9% methyl ethyl ketone and saline as mobile phases, respectively. Human serum albumin (HSA, 1%)-impregnated ITLC-SG (Gelman Science, Inc., Ann Arbor, MI, USA) strips were used as the stationary phase and ethanol:NH4OH:water (2:1:5 v/v) as the mobile phase to separate radiocolloids, which remained at the base,