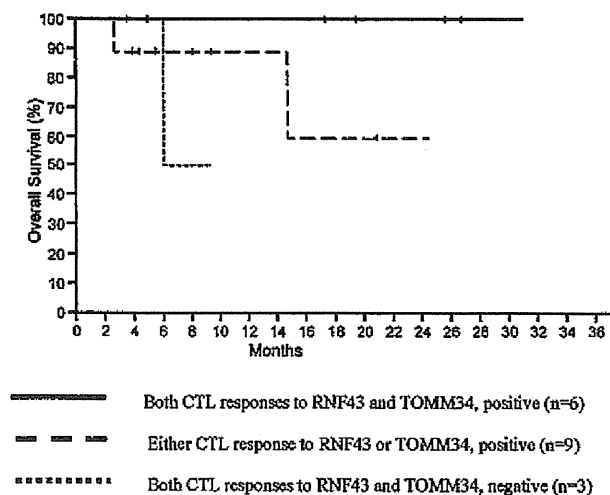FOLFOX (combination of oxaliplatin, 5-FU, and LV) chemotherapy for metastatic CRC patients. Among 26 patients, 13 patients had a partial response, 12 patients had stable disease, and 1 patient had progressive disease. The median progression-free survival has not been calculated (Shoichi Hazama, personal communication).

Both CTL responses to RNF43 and TOMM34, positive (n=6)

Either CTL response to RNF43 or TOMM34, positive (n=9)

Both CTL responses to RNF43 and TOMM34, negative (n=3)

Fig. (2). Overall survival of patients with specific T cell responses to RNF43 and TOMM34.
Patients with responses to both RNF43 and TOMM34 had the best survival. Patients having no responses had worst survival. Patients with one response had intermediate survival.

RATIONALE OF COMBINATION THERAPY

Cancer is an extremely complex and heterogeneous disease that robustly resists host-defense systems and therapeutic efforts. The loss of MHC class I expression is a major mechanism of tumor cell escape from immune surveillance, whereas the appearance of multidrug resistance is the major mechanism of tumor cell resistance to chemotherapy. One approach to overcome the resilience of cancer is the design of a new combination therapy in which each modality imposes independent selective pressure to the acquired mutations of cancer [17].

Chemo-Immunotherapy in CRC

The combined chemo-immunotherapy approach has been criticized on the grounds that chemotherapy is immunosuppressive. This opinion is based on the fact that most cytotoxic drugs can kill granulocyte precursors in bone marrow and thus induce leucopenia, which is associated with the occurrence of bacterial and mycotic infection. However, there is no evidence that cytotoxic chemotherapy affects the antigen-specific CTL response. Recently, Correale et al. [30] reported that the antigen-specific killing ability of human CTL lines in vitro is not affected by FU, oxaliplatin, or gemcitabine (GEM) if exposure to these drugs does not occur during the stimulation phase. Moreover, they found that chemotherapy (1) up-regulated tumor-associated antigen expression, including CEA or other target molecules such as thymidylate synthase (TS); (2) down-regulated tumor cell resistance to the death signals induced by tumor antigen-

specific cytotoxic T lymphocytes; (3) reduced the percentage of PBMCs containing immune-suppressive regulatory T cells (CD4+CD25+T reg) and the number of cells expressing the FAS receptor (CD95); and (4) induced the complete restoration of the CD4/CD8 T cell ratio, which is often reduced in advanced cancer patients showing a progressively deteriorating immune response [31].

HLA Loss or Down-Regulation in Cancer Progression

For successful CTL-based immunotherapy, it is essential to eliminate the loss of major histocompatibility complex (MHC) class I on cancer cells. A large population (30 – 60%) of cancer cells do not express MHC class I molecules, which are crucial for CTL-mediated elimination of cancer cells [32]. This problem, however, could be overcome by the combined use of another type of peptide vaccine, such as peptide of VEGFR1, or VEGFR2 [33], and either chemotherapy [31] or cytokine therapy capable of activating innate immunity including natural killer cells and macrophages. From this viewpoint, the development of an effective vaccine against tumor angiogenesis is suitable, because endothelial cells are genetically stable, do not down-regulate HLA class I molecules, and are critically involved in the progression of a variety of tumors. Furthermore, the CTLs could directly cause damage to the endothelial cells without penetrating any other tissue, and the lysis of even low numbers of endothelial cells within tumor vasculature may result in the destruction of vessel integrity, leading to the inhibition of many tumor cells. The results of a phase I study of multiple peptide vaccination including VEGFR1 and VEGFR2 in combination with FOLFOX chemotherapy for patients with metastatic CRC by Hazama et al. are anticipated.

FUTURE PERSPECTIVES

Numerous studies of vaccination in CRC patients have been performed. Antigen-specific responses were induced to some extent, depending on the individual immunizing methods in the trials; however, the clinical responses were marginal. In a meta-analysis by Nagorsen et al. [34], the objective response rate was only 0.9% for 527 CRC patients treated with active specific immunotherapy in 32 different studies. There are several possible approaches to improve the poor clinical outcome of vaccine immunotherapy in CRC.

Adjuvant Setting

Despite the nearly complete lack of a clinical response in patients with advanced colorectal cancer, a few studies have shown that adjuvant active specific immunotherapy may be beneficial in subgroups of patients after CRC resection [35, 36]. As we do not expect vaccination in patients with a high tumor burden to be highly clinically effective, we may be able to obtain a better impact on clinical outcome from the adjuvant setting. Recently, we started a randomized trial of CRC-specific peptides (RNF43, TOMM34) in combination with UFT/ LV chemotherapy as adjuvant immunochemotherapy in stage III colorectal cancer patients.

Helper-Peptide Vaccines

Cancer vaccine therapy first focused on the activation of CD8+ cytotoxic T cells (CTLs), which eradicate tumors *in*

vivo. Although many investigators approached this problem by using MHC class I-binding peptides, this approach has been hampered by strong immunosuppression and unknown immune-escape mechanisms in tumor-bearing hosts. CD4+ T cells are crucial for the induction of effective antitumor immunity. In particular, the introduction of T helper type 1 (Th1)-dominant immunity in tumor-bearing hosts is critically important to overcome immunosuppression and induce fully activated tumor-specific CTLs. Nishimura *et al.* [37] reported that adoptively transferred tumor-specific Th1 cells exhibited strong antitumor activity *in vivo*. Moreover, they established cancer-specific helper T cell lines from healthy donors by using cancer antigen NY-ESO-1 derived from overlapping 15-mer synthetic peptides bound to HLA-DR molecules [38]. This method is anticipated to be a new cancer vaccine therapy that elicits a cancer-specific helper T cell response in cancer patients. In collaboration with the Nishimura group, we recently started a helper-peptide vaccine study with Survivin or MAGE-A4 antigen-derived helper peptides for the treatment for advanced CRC patients.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

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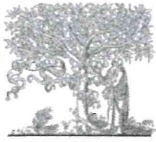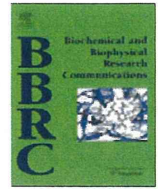
Switching addictions between HER2 and FGFR2 in HER2-positive breast tumor cells: FGFR2 as a potential target for salvage after lapatinib failure

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ABSTRACT

Agents that target HER2 have improved the prognosis of patients with *HER2*-amplified breast cancers. However, patients who initially respond to such targeted therapy eventually develop resistance to the treatment. We have established a line of lapatinib-resistant breast cancer cells (UACC812/LR) by chronic exposure of *HER2*-amplified and lapatinib-sensitive UACC812 cells to the drug. The mechanism by which UACC812/LR acquired resistance to lapatinib was explored using comprehensive gene hybridization. The *FGFR2* gene in UACC812/LR was highly amplified, accompanied by overexpression of FGFR2 and reduced expression of HER2, and a cell proliferation assay showed that the IC_{50} of PD173074, a small-molecule inhibitor of FGFR tyrosine kinase, was 10,000 times lower in UACC812/LR than in the parent cells. PD173074 decreased the phosphorylation of FGFR2 and substantially induced apoptosis in UACC812/LR, but not in the parent cells. FGFR2 appeared to be a pivotal molecule for the survival of UACC812/LR as they became independent of the HER2 pathway, suggesting that a switch of addiction from the HER2 to the FGFR2 pathway enabled cancer cells to become resistant to HER2-targeted therapy. The present study is the first to implicate FGFR in the development of resistance to lapatinib in cancer, and suggests that FGFR-targeted therapy might become a promising salvage strategy after lapatinib failure in patients with HER2-positive breast cancer.

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1. Introduction

Breast cancer is the second most frequent malignancy worldwide, and the prognosis of patients with metastatic disease still remains very poor, despite intensive research and drug development [1]. Amplification of the human epidermal growth factor receptor 2 (HER2) gene has been detected in 20–30% of human breast cancers, driving tumor development and being associated with a poor outcome [2]. HER2 forms dimers to become active, and its dimerization partners are the epidermal growth factor receptor (EGFR), HER2 itself, and HER3 in most cases. Since EGFR is a molecule frequently expressed in HER2-positive breast cancer, interaction between EGFR and HER2 could be important for the maintenance

of oncogenesis [3]. Thus, targeting HER2 and EGFR together appears to be a promising therapeutic strategy for patients with *HER2*-amplified breast cancer, and multi-targeted small-molecule inhibitors such as lapatinib, BIBW2992 and AZD8931, directed against EGFR family members, have been developed for this purpose. Lapatinib binds to the ATP binding sites of EGFR and HER2, thus inhibiting their tyrosine kinase activity [4].

Acquired resistance to HER2-targeted drugs is one of the major obstacles to further improvement of clinical outcomes in this field, and research efforts have been focused on clarifying the mechanisms by which cancer cells acquire resistance to lapatinib. Several mechanisms of resistance to trastuzumab, a humanized monoclonal antibody against HER2, have been proposed, such as the presence of a truncated form of HER2 without an extracellular domain, loss of PTEN, and *PIK3CA* mutations in pre-clinical models, and such mechanisms may also have some implications for the lapatinib resistance phenotype [5–7]. In addition, overexpression of AXL, a receptor type kinase, has been reported to be a critical player for bypassing lapatinib-elicited HER2-PI3K-Akt signaling and conferring resistance to the drug in a breast cancer cell line [8].

Fibroblast growth factor receptor 2 (FGFR2) is a member of the FGFR tyrosine kinase family, and consists of 4 receptors and 23 ligands [9]. Ligand binding leads to FGFR2 dimerization, autophosphorylation, and activation of signaling components including Akt and Erk kinases. Amplification and overexpression of the *FGFR2* gene is observed in gastric cancer and breast cancer [9], and single-nucleotide polymorphisms (SNPs) of the *FGFR2* gene are associated with a higher risk of sporadic breast cancer [10]. These features suggest that *FGFR2* may have an oncogene-like character, and be capable of transforming normal cells. This gene could act as a driving force for transformation of cancer cells into a further malignant phenotype, and constitute a potential target of treatment in cancer patients whose tumors express the protein.

Here we report that subpopulations of cells with *FGFR* gene amplification play a pivotal role in development of resistance to lapatinib in HER2-positive breast cancer.

2. Materials and methods

2.1. Cell culture and reagents

A human breast cancer cell line, UACC812 was obtained from the American Type Culture Collection (Manassas, VA), and cultured under a humidified atmosphere of 5% CO_2 at 37 °C in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum. Gefitinib was obtained from Kemprotec Ltd. (UK). Lapatinib was obtained from Chemietek (Indianapolis, IN). PD173074 was purchased from Sigma (St. Louis, MO).

2.2. Generation of a lapatinib-resistant line and floating line from UACC812

The UACC812 cells were grown initially in medium containing 0.01 μM lapatinib, and the concentration was gradually increased up to 1 μM over the following 8 months to establish lapatinib-resistant cell lines (UACC812/LR).

2.3. Array-based comparative genomic hybridization

The Genome-wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA) was used to perform array-CGH on genomic DNA from each of the cell lines, in accordance with the manufacturer's instructions. A total of 250 ng of genomic DNA was digested with the restriction enzymes Nsp I and Sty I in independent parallel reactions (SNP6.0), ligated to the adaptor, and amplified using PCR with a universal primer and TITANIUM Taq DNA Polymerase (Clontech). The PCR products were quantified, fragmented, end-labeled, and hybridized onto a Genome-wide Human SNP Array 6.0. After washing and staining in Fluidics Station 450 (Affymetrix), the arrays were scanned to generate CEL files using the GeneArray Scanner 3000 and GeneChip Operating Software ver.1.4. In the array-CGH analysis, sample-specific changes in copy number were analyzed using Partek Genomic Suite 6.4 software (Partek Inc., St. Louis, MO).

2.4. Growth assay in vitro

Cells were cultured in 96-well flat-bottomed plates for 24 h before exposure to various concentrations of drugs for 72 h. TetraColor One (5 mM tetrazolium monosodium salt and 0.2 mM 1-methoxy-5-methyl phenazinium methylsulfate; Seikagaku, Tokyo, Japan) was then added to each well, and the cells were incubated for 3 h at 37 °C before measurement of absorbance at 490 nm with a Multiskan Spectrum instrument (Thermo Labsystems, Boston, MA). Absorbance values were expressed as a percentage relative to untreated cells, and the concentration of tested drugs resulting in 50% growth inhibition (IC_{50}) was calculated using the Prism program (GraphPad, San Diego, CA).

2.5. Cell death assay

After incubation, cells were harvested by trypsinization and resuspended in a solution of 1 μg/mL propidium iodide in PBS, then immediately acquired on the FL3 channel of a flow cytometer. The population of propidium iodide-positive cells was considered dead, whereas the propidium iodide-negative population was considered viable.

2.6. Immunoblot analysis

Cells were washed twice with ice-cold PBS and then lysed with 1× Cell Lysis Buffer (Cell Signaling Technology) containing 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, and leupeptin (1 μg/ml). The protein concentration of cell lysates was determined with a BCA protein assay kit (Thermo Fisher Scientific); and equal amounts of protein were subjected to SDS–PAGE on a 4–12% gradient gel. The separated proteins were transferred to a PVDF membrane, which was then incubated with Blocking One solution (Nakarai Tesque, Kyoto, Japan) for 20 min at room temperature before incubation overnight at 4 °C with primary antibodies, including those against phosphorylated FGFR, phosphorylated EGFR(Y1086), phosphorylated HER2(Y1221/1222), EGFR, FGFR1, FGFR3, FGFR4, phosphorylated AKT, AKT, ERK, PARP, caspase-3 (Cell Signaling Technology, Danvers, MA), HER2 (Millipore), FGFR2 (Bek) and phosphorylated ERK (Santa Cruz Biotechnology) or β-actin (1:5000 dilution, Sigma). The membrane was then washed with PBS containing 0.05% Tween 20 before incubation for 1 h at room temperature with horseradish peroxidase-conjugated antibody against rabbit immunoglobulin G (Sigma). Immune complexes were finally detected using ECL Western blotting detection reagents (GE Healthcare, Little Chalfont, UK). The RTK array was purchased from R & D Systems (Minneapolis, MN) and used in accordance with the manufacturer's instructions.

2.7. Assay of phospho-FGFR2 activity

The activity of p-FGFR2 in cell lysates was measured using ELISA in accordance with the manufacturer's procedures (Human phosphor-FGFR2 Duoset; R & D Systems). The lysates were prepared as described above. All samples were run in triplicate assays. Color intensity was measured at 450 nm using a spectrophotometric plate reader. Growth factor concentrations were determined by comparison with standard curves.

2.8. FGFR2 gene silencing using small interfering RNA

Cells were plated at 50–60% confluence in six-well plates or 25 cm^2 flasks and then incubated for 24 h before transient transfection for 48 h with small interfering RNAs (siRNAs) mixed with Lipofectamine reagent (Invitrogen, Carlsbad, CA). A siRNA specific for FGFR2 mRNA and a nonspecific siRNA (control) were obtained from Nippon EGT (Toyama, Japan). The cells were then subjected to flow cytometry and immunoblot analysis.

2.9. Immunohistochemistry (IHC)

Paraffin-embedded tissue samples were cut at a thickness of 4 μM and examined on coated glass slides, after labeling with antibodies directed against the following using the ChemMate ENVISION method (DakoCytomation, Glostrup, Denmark). Endogenous

peroxidase activity was inhibited by incubating the slides in 3% H_2O_2 for 20 min. FGFR2 (C-17, Santa Cruz Biotechnology) antigen retrieval was done by microwaving for 10 min in Target Citrate Solution (pH 6.0). Each slide was incubated overnight with the antibody at 4 °C. For staining detection, the ChemMate ENVISION method was used with DAB as the chromogen. The expression of FGFR2 protein in the cell membrane and cytoplasm was investigated in detail. FGFR2 expression was classified into three categories: score 0, no staining at all; or membrane expression in <10% of cancer cells; score 1+, faint/barely perceptible partial membrane expression in ⩾10% of cancer cells; score 2+, weak to moderate expression on the entire membrane in ⩾10% of the cancer cells; score 3+, strong expression on the entire membrane in ⩾10% of cancer cells. All IHC studies were evaluated by two IHC-experienced reviewers (K.A. and J.T.) who were blind to the conditions of the patients. Consistent results were obtained in 14 out of 16 samples, and two IHC samples without consistency were subjected to scoring by a third reviewer who was also blinded to the clinical information and scores assigned previously by the other two reviewers. Then, the majority scores were employed as the final results.

2.10. Study population and survival analysis

All patients received lapatinib between 2009 and 2010 at Kinki University School of Medicine. Sixteen tumors from a series of 13 patients diagnosed as having HER2-positive metastatic breast cancer were collected from the files of the Pathology Department, Kinki University School of Medicine, covering the period between 2009 and 2010. The HER2 status was considered positive if the local institution reported grade 3+ staining intensity (on a scale of 0–3) by means of IHC analysis or grade 2+ staining intensity by means of IHC analysis with gene amplification on fluorescence in situ hybridization. Details of the patients' clinical characteristics, including age, hormone status, prior therapy, and tumor response were obtained from chart review by an independent reviewer who was unaware of the results of IHC analysis. Tumor responses were evaluated after chemotherapy according to the Response Evaluation Criteria for Solid Tumors (RECIST). Four sites of metastasis were included. Any material that had been poorly fixed and/or had low cellularity was rejected. Paraffin-embedded tissues were obtained, and histologic examination of slides stained with hematoxylin-eosin and saffron was carried out by a specialist. All patients provided written informed consent for collection of their tissue material and clinical data for research purposes, and the tissue procurement protocol was approved by the institutional review board.

Progression-free survival was defined as the time between the onset of chemotherapy and the date when disease progression began. Patients without progression were regarded as censored at the date of the last follow-up. Curves for progression-free survival were estimated by the Kaplan–Meier method, and differences in survival functions were compared by the log-rank test.

All tests were two-sided, and differences at $P < 0.05$ were considered statistically significant. All the statistical analyses were conducted using JMP version 8 software (SAS Institute Inc., Cary, NC).

2.11. Fluorescence in situ hybridization

The gene copy number per cell for HER2 was determined by fluorescence in situ hybridization (FISH) with the use of HER2/neu (17q11.2–q12) Spectrum Orange and CEP17 (chromosome 17 centromere) Spectrum Green probes (Vysis; Abbott, Des Plaines, IL). Gene amplification was defined as a mean HER2/chromosome 17 copy number ratio of >2.

2.12. Statistics

Experimental values were expressed ±SE. Statistical comparison of mean values was done using Student's t test.

3. Results

### 3.1. Establishment of lapatinib-resistant breast cancer cells

The UACC812 cells were grown initially in medium containing 0.01 μM lapatinib, and the concentration was gradually increased to 1 μM over the following 8 months to establish lapatinib-resistant cell lines (UACC812/LR). Cell growth assays were performed for the UACC812 cells and the UACC812/LR cells with various doses of lapatinib and gefitinib, as indicated in Fig. 1A, and the IC_{50} values were determined (Fig. 1A inset). UACC812/LR cells were resistant to lapatinib and gefinitib in comparison with the parent cells, the IC_{50} values for lapatinib (4.2433 ± 0.5066 μM) and gefitinib (11.1300 ± 0.5474 μM) being 42 times and 6 times higher than those in UACC812 (0.1006 ± 0.0053 μM and 1.9223 ± 0.3744 μM), respectively, but no differences were seen between the two cell lines in terms of the IC_{50} values for cisplatin and 5-FU (Fig. 1A inset), suggesting that chronic exposure of the UACC812 cells to lapatinib had induced resistance specific to EGFR or HER2 inhibitors.

3.2. FGFR2 gene amplification in UACC812/LR

To overview the chromosomal divergences between the parent cell line and its derivative, comprehensive gene hybridization (CGH) analyses were performed as described in Section 2. This revealed that the UACC812/LR cells harbored an amplification of the fibroblast growth factor receptor 2 (FGFR2) gene, the gene copy number in UACC812/LR being approximately 20 times that in UACC812 (Fig. 1B). Lysates of the parent and the derivative cells were subjected to Western blotting-based high-throughput analysis for expression of various receptor type kinases (RTK), and a dramatic increase in the expression of FGFR2 was observed in UACC812/LR relative to UACC812 (Fig. 1C). In contrast, the expression of HER2 was reduced in UACC812/LR in comparison to the parent cells (Fig. 1C upper panel). HER2-FISH analysis revealed that the HER2 gene amplification was present in UACC812 cells, but not in UACC812/LR cells (Fig. 1C lower panel). To examine the role of FGFR2 in the growth of the parent cells and their derivative, an FGFR-TKI, PD173074, was utilized, and cell growth assays were performed for UACC812 and UACC812/LR treated with various concentrations of PD173074. UACC812/LR was more sensitive than UACC812 to PD173074, the IC_{50} (0.00121 ± 0.0034 μM) being 10,000 times lower than that for the parent cells (10.3373 ± 1.6629 μM), indicating that UACC812/LR cells had acquired dependency on the FGFR2 pathway, whereas FGFR2 played no role in the cell growth of UACC812 (Fig. 1D and inset).

3.3. UACC812/LR shows high phosphorylation of FGFG2 and undergoes apoptosis upon exposure to a FGFR tyrosine kinase inhibitor

To further evaluate the findings of the RTK arrays and cell growth assays, Western blotting was performed for biochemical profiling of these cell lines. Overexpression of phosphorylated FGFR2 (p-FGFR2) and native FGFR2, and downregulation of p-HER2 and p-EGFR in UACC812/LR cells relative to the parent cells were observed (Fig. 2A). The two cell lines were treated with lapatinib (1 μM) or PD173074 (0.1 and 1 μM) for 24 h, and the cell lysates were then subjected to Western blot analysis. The basal levels of p-EGFR, p-HER2 and native HER expression were
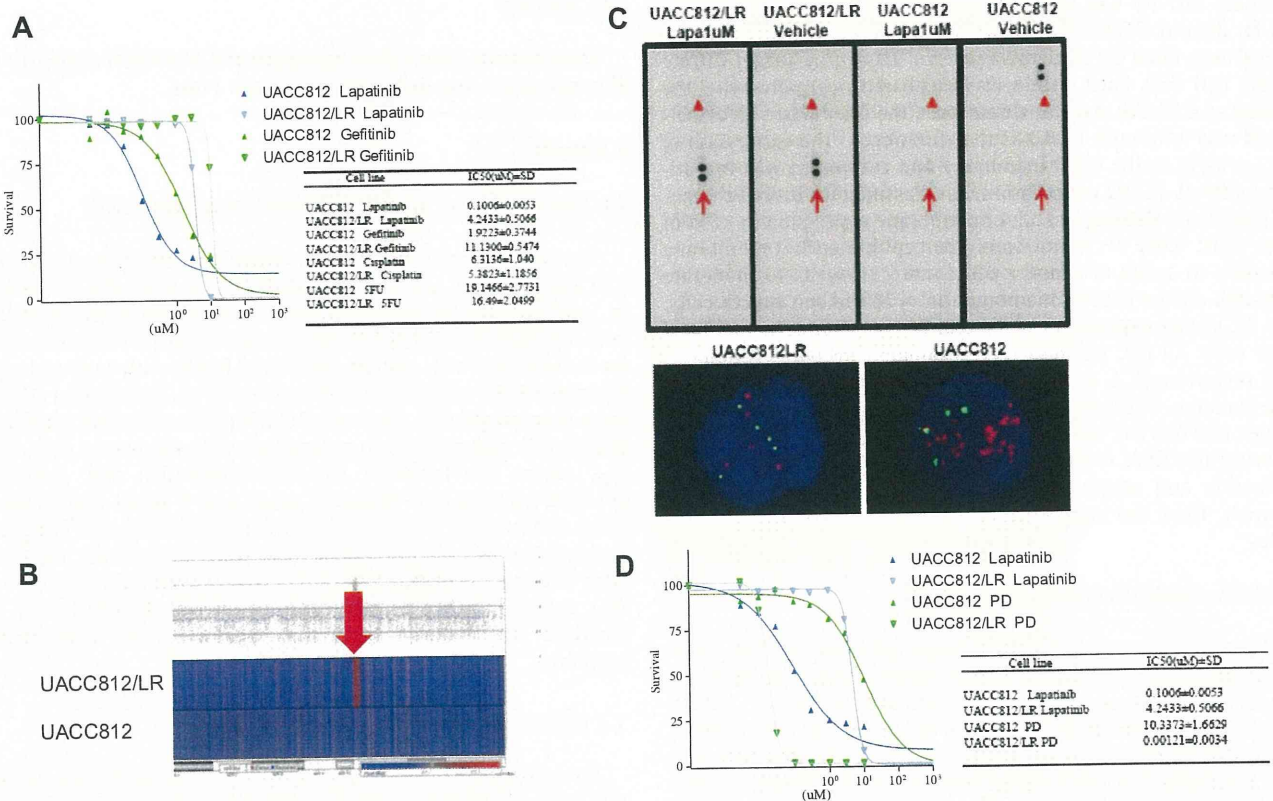
Fig. 1. Lapatinib-resistant cancer cells harbor *FGFR2* gene amplification, and are preferentially sensitive to an FGFR-TKI. (A) Cell growth assays were performed using UACC812 cells and their derivative, UACC812/LR cells, treated with lapatinib, gefitinib, cisplatin or 5-FU for 72 h, and IC$_{50}$ values are shown in the inset. Results represent the mean ± SE of three experiments performed in triplicate. (B) *FGFR2* gene amplification detected in UACC812/LR cells. Comprehensive gene hybridization analysis revealed that the gene on chromosome 10q26 was highly amplified in UACC812/LR relative to its parent cell line. (C) Lysates from UACC812 cells and UACC812/LR cells treated with vehicle or 1 μM lapatinib for 6 h were subjected to Western blotting-based high-throughput analysis for RTKs. Arrows and arrowheads indicate signals for FGFR2 and HER2, respectively. Left column: UACC812/LR cells treated with 1 μM lapatinib; Second column from left: UACC812/LR treated with vehicle; Second column from right: UACC812 cells treated with 1 μM lapatinib; Right column: UACC812 cells treated with vehicle. Images of HER2-FISH analyses in UACC812 and UACC812/LR are shown. (D) Cell growth assays were performed using UACC812 cells and UACC812/LR cells with lapatinib or PD173074 at various doses for 72 h. IC$_{50}$ values are shown in the inset. Results represent the mean ± SE of three experiments performed in triplicate.

decreased, and those of p-FGFR and native FGFR2 were dramatically increased in UACC812/LR cells (Fig. 2A). Lapatinib inhibited the expression of p-HER2 and p-EGFR accompanied by downregulation of p-Akt and p-Erk in UACC812 cells, but no inhibition of the phosphorylation of these signal components was observed in UACC812/LR (Fig. 2A). On the other hand, PD173074 did not affect the level of phosphorylated Akt or Erk in the parent cells, but inhibited that of p-FGFR along with p-Akt and p-Erk in UACC812/LR cells (Fig. 2A). Cleaved poly (ADP-ribose) polymerase (PARP) and caspase 3 as markers of apoptosis were increased in UACC812 and UACC812/LR after treatment with lapanib or PD173074, respectively, suggesting that the parent cells and their derivative were dependent on the different pathways for survival (Fig. 2A). Since a pan-antibody against p-FGFR was utilized in the Westen blotting to detect the pharmacological activity of PD173074, we further examined p-FGFR2 in an ELISA assay using a specific antibody against p-FGFR2 in UACC812 and UACC812/LR cells treated with lapatinib or PD173074 (Fig. 2B). We found that the basal level of p-FGFR2 was increased in UACC812/LR relative to the parent cells, and that phosphorylation was inhibited by PD173074 but not by lapatinib. Induction of cell death with lapatinib and/or pharmacological or genetic abrogation of FGFR2 was then measured in UACC812 and UACC812/LR cells (Fig. 2D). Cell death was induced in UACC812 cells treated with lapatinib but not in those treated with PD173074 (Fig. 2D left panel) or si-RNA for FGFR2 (Fig. 2D right panel). There were no increases in the percentage of cell

death induced by lapatinib upon addition of PD173074 or si-RNA for FGFR2 in UACC812 cells or UACC812/LR (Fig. 2D left panel and right panel). Nonetheless, PD173074 and si-RNA for FGFR2 dramatically induced cell death in UACC812/LR cells (Fig. 2D left panel and right panel). Validation of the biochemical effects of si-RNA treatment on FGFR2 is shown in Fig. 2C. Overexpression of FGFR2 was observed in UACC812/LR cells relative to UACC812 cells, and treatment with si-RNA for FGFR2 reduced the expression of FGFR2, accompanied by inhibition of p-Akt and p-Erk in UACC812/LR cells (Fig. 2C). Cleaved PARP and caspase 3 were induced in UACC812 cells and UACC812/LR cells treated with lapatinib and/or si-RNA for FGFR2, respectively (Fig. 2C). Together, these findings suggested that UACC812/LR cells had become addicted to the FGFR2 pathway for survival in the absence of the activated HER2 pathway during the development of resistance to lapatinib.

3.4. High expression of FGFR2 in tumor specimens is associated with poor response to lapatinib

To further evaluate the role of FGFR2 in a clinical setting, we examined tissue specimens obtained from 13 consecutive patients with metastatic HER2-positive breast cancer treated with lapatinib between 2009 and 2010 at our institution. The median age of the patients was 60 years (35–69 years) and the median follow-up time after administration of lapatinib was 275 days (42–358 days). All the patients had been treated with lapatinib, and the
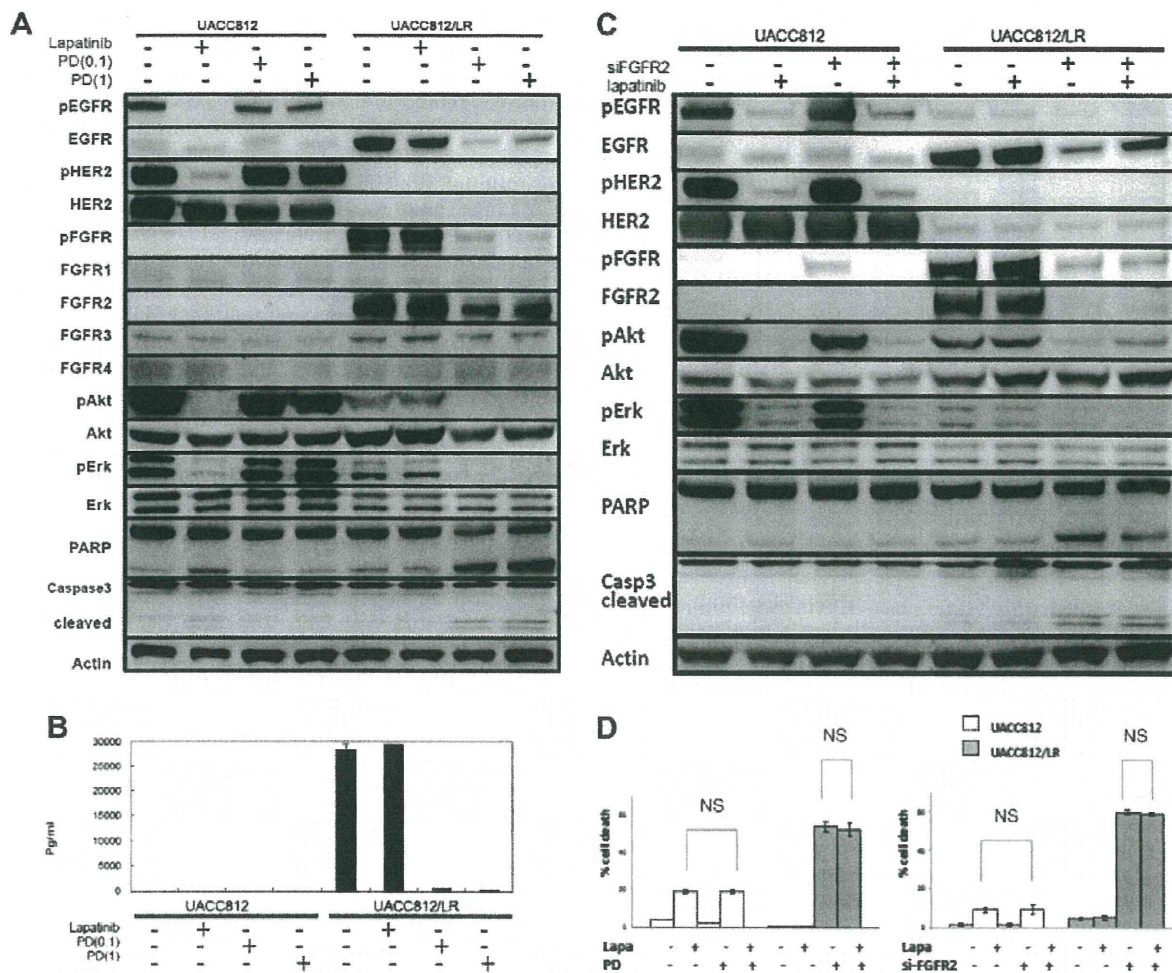
Fig. 2. FGFR2 is active in the lapatinib-resistant cell line, UACC812/LR, but not in the parental cells. (A) UACC812 cells and UACC812/LR cells were treated with 1 μM lapatinib, or 0.1 or 1 μM PD173074 for 24 h, as described in Section 2. Lysates were subjected to Western blotting with the indicated antibodies. (B) Phospho-FGFR2 levels were measured in UACC812 cells and UACC812/LR cells treated with vehicle, 1 μM lapatinib, or 0.1 or 1 μM PD173074 using an ELISA-based assay. Results represent the mean ± SE of three experiments performed in triplicate. (C) UACC812 cells and UACC812/LR cells were treated with 1 μM lapatinib and/or si-RNA for FGFR2 for 24 h after completion of transfection. Lysates were subjected to Western blotting with the indicated antibodies. (D) UACC812 cells and UACC812/LR cells were treated with 1 μM lapatinib and/or 0.1 μM PD173074 for 48 h (left panel) or with 1 μM lapatinib and/or si-RNA for FGFR2 for 48 h after completion of transfection (right panel). The cells were harvested and subjected to flow cytometry analysis to assess the extent of cell death. Results represent the mean ± SE of three experiments performed in triplicate. NS, not statistically significant.

Table 1
Clinicopathological features of HER2 positive MBC.

Patients #	Age	Primary hormone receptor status	Prior therapy	Response[*]	FGFR2 expression
1	52	ER+, PgR+	H, T, A	SD	0
2	53	ER+, PgR–	H, A	SD	1+
3	47	ER+, PgR–	H, T, A	PR	1+
4	60	ER+, PgR–	H, T	SD	1+
5	69	ER–, PgR–	H, T, A	PD	2+
6	67	ER–, PgR–	H	NE	1+
7	52	ER+, PgR–	H, T	PD	0
8	47	ER–, PgR–	H, T, A	SD	1+
9	35	ER+, PgR+	H , T, A	PR	1+
10	65	ER–, PgR–	H, T, A	NE	0
11	61	ER–, PgR–	H , T, A	PD	2+
12	69	ER+, PgR+	H, T, A	PD	2+
13	59	ER–, PgR–	H, T	PR	0

MBC, metastatic breast cancer; ER, estrogen receptor; PgR, progesterone receptor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; H, Herceptin; T, Taxanes; A, Anthracycline.
[*] Response to lapatinib-containing regimens.

clinicopathological features including IHC scores of FGFR2 in tumor specimens are summarized in Table 1. Time to progression (TTP) while receiving the treatment was plotted using Kaplan–Meier curves stratified by FGFR2 expression (score 0, 1 vs. 2, 3, Fig. 3), and the patients with FGFR2-overexpressing tumors had significantly poor survival ($P = 0.0082$), suggesting that FGFR2 may play at least a partial role in the development of resistance to lapatinib, probably through selection of FGFR2-overexpressing tumor cells.

4. Discussion

Several models have been proposed to account for the clinical resistance to HER2-targeted therapies including *PIK3CA* gene mutation and *AXL gene amplification* [5–8]. Gene sequence analyses revealed that UACC812 and UACC812/LR did not harbor *PIK3CA* gene mutation (data not shown), and Western blotting showed that UACC812/LR cells do not express AXL (data not shown), indicating that these two molecules are not causative factors for acquired resistance to lapatinib in UACC812/LR. Instead, the
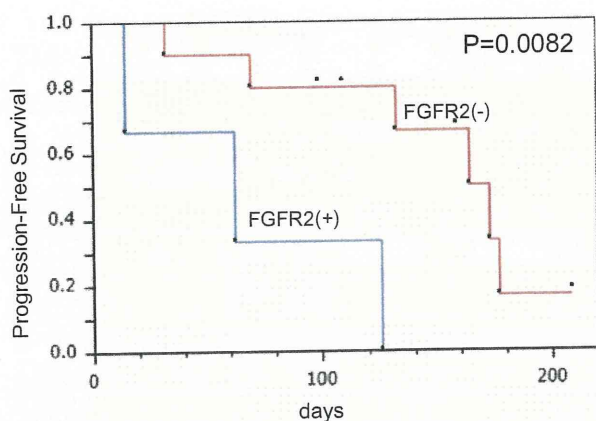
Fig. 3. Kaplan–Meier curves illustrating associations between protein expression and progression-free survival since the start of lapatinib. Survival curves are plotted as graphs according to FGFR2 expression level. The *P*-value was calculated using Log-rank test.

present model revealed amplification of *FGFR2* in lapatinib-resistant cells, and activation of FGFR2 substantially contributed to survival of the cells.

There is compelling evidence for deregulated FGF signaling in the pathogenesis of many cancers that originate from different tissue types. Aberrant FGF signaling can promote tumor development by directly driving cancer cell proliferation and survival. The underlying mechanism driving FGF signaling is largely tumor-specific, and can be attributed to genomic *FGFR* alterations that drive ligand-independent receptor signaling. Mutations of *FGFR2*, which are frequently extracellular, have been described in 12% of endometrial carcinomas [11]. *FGFR2*-mutant endometrial cancer cell lines are highly sensitive to FGFR tyrosine kinase inhibitors, suggesting oncogenic addiction of the cancer cells to the activated form of mutant FGFR [12]. In this study, gene sequencing analysis of *FGFR2* in UACC812 cells and UACC812/LR cells revealed no mutations, but gene amplification was noted in UACC812/LR in comparison with UACC812. Amplifications of *FGFR2* have been reported in approximately 10% of gastric cancers, and have been associated with poor prognosis [13]. Gastric cancer cell lines with *FGFR2* amplifications show ligand-independent signaling and are highly sensitive to FGFR inhibitors [13]. Inhibition of FGFR2 substantially induced cell death in UACC812 cells/LR harboring gene amplification of *FGFR2*, but not in UACC812 cells, and this was consistent with the above reports.

UACC812/LR cells showed loss of *HER2* amplification after chronic exposure to lapatinib, being reminiscent of clinical observations. Loss of *HER2* amplification following trastuzumab-based neoadjuvant systemic therapy has been reported in patients with residual breast cancers [14]. One third of patients with significant residual disease showed loss of *HER2* amplification, and this change was associated with poor relapse-free survival. We speculate that these residual tumors after trastuzumab-based therapy may have harbored alternative driving genes to support further tumor development under selection pressure with trastuzumab, and our model using UACC812/LR cells recapitulated the clinical loss of HER2 resulting from HER2-targeted therapies, although lapatinib was used instead of trastuzumab in this study.

Together, these findings suggest that FGFR2 may be a key molecule in the development of resistance to lapatinib in HER2-positive breast cancer through selection of cells with a growth advantage and improved survival, and that FGFR-targeted therapy may be a promising strategy for breast cancer patients in whom treatment with lapatinib has failed. Further clinical studies using a larger set of tumor specimens should be performed to confirm our findings in a small set of clinical samples, and development of FGFR-targeted therapy is warranted to clarify the role of FGFR in resistance to HER2-targeted medicines.

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Case Reports in Dermatology

Transient Effectiveness of an Oral 5-Fluorouracil Derivative, S-1, for Epirubicin, Cyclophosphamide and Paclitaxel Refractory Skin Metastases from Possible Occult Breast Cancer in a Male

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Key Words

Occult breast cancer · Skin metastases · S-1

Abstract

Recent chemotherapies for skin metastases from breast cancer have shown to be effective for regression, disappearance, and favorable quality of life. We describe the case of a 76-year-old male showing transient effectiveness with an oral 5-fluorouracil derivative, S-1 (tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate), for epirubicin, cyclophosphamide and paclitaxel refractory skin metastases from possible occult breast cancer. The male patient was initially diagnosed as having lymph node metastases in the left axilla as possible occult breast cancer. The skin metastases developed after chemotherapy with a combination of epirubicin and cyclophosphamide, subsequent chemotherapy with paclitaxel, and radiotherapy. Chemotherapy with paclitaxel was resumed for skin metastases, but it was not effective. Alternative chemotherapy with the oral agent S-1 was administered. The skin metastases completely disappeared after the second course, but recurred at the end of the third course. This case suggests that S-1 may be a candidate for chemotherapy for skin metastases from occult breast cancer in males.

Introduction

Breast cancer in a male is very rare, comprising less than 1% of all breast cancers and less than 1% of all cancers in men [1, 2]. The occurrence of occult breast cancer in a male

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is even rarer, although some cases have been reported [3, 4]. Here, we report a male having epirubicin, cyclophosphamide and paclitaxel refractory skin metastases from possible occult breast cancer. The skin metastases transiently disappeared completely following a chemotherapy with an oral 5-fluorouracil (5-FU) derivative, S-1 (tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate; Taiho Pharmaceutical Co. Ltd., Tokyo, Japan).

Case Report

A 76-year-old Japanese man presented to our department with asymptomatic eruptions. The initial examination revealed asymptomatic erythematous patches and linear macules, which were gradually increasing in size and number on the left chest and back (fig. 1a). The patient had had an edematous swelling on the left arm and a palpable lymph node on the left axilla 1.5 years earlier. Lymph nodes, except the left axilla, had not been palpable. A chest X-ray had been clear. A positron emission tomography (PET)/computed tomography (CT)-scan of the entire body had shown only enlarged lymph nodes in the left axilla and had not depicted any other lesions. The lymph nodes in the left axilla had been excised surgically. Tumor cells were detected in 9 out of 30 lymph nodes. Immunohistochemical staining showed positive reaction to gross cystic disease fluid protein-15 (GCDFP-15) and mammaglobin, and negative reaction to human epithelial growth factor receptor type 2 (HER2), estrogen receptor (ER), and progesterone receptor (PgR). The patient had been diagnosed as having lymph node metastases from possible occult breast cancer. He had received chemotherapy consisting of epirubicin 25 mg/m² and cyclophosphamide 100 mg/body once a week for 18 courses, following chemotherapy with paclitaxel 80 mg/m² once a week for 14 courses, and then he had been given radiotherapy consisting of electron radiation, 2 Gy per irradiation (total 30 times, 60 Gy), on the left upper chest, axilla and back.

Histopathological examination of a biopsy specimen revealed an extensive invasion of the dermal lymphatics by groups and cords of tumor cells (fig. 1b). The cells were similar to those in the lymph nodes in the left axilla. Morphologically, they were irregular in size and shape, with large, pleomorphic and hyperchromatic nuclei (fig. 1d). Immunohistochemical staining showed positive reaction to GCDFP-15, and negative reaction to mammaglobin, HER2, ER and PgR. The erythematous patches and linear macules were diagnosed to be skin metastases from possible occult breast cancer. Systemic examination showed no enlarged lymph nodes or focal lesions except skin involvement.

Chemotherapy with paclitaxel was resumed. The regimen was the same as in the previous treatment (80 mg/m² once a week). At the end of the 5 courses, we concluded that the chemotherapy was not effective for the metastatic cancer. The erythematous patches and linear macules grew into a painful large dark reddish patch and necrotic nodules (fig. 1c).

An alternative chemotherapy with S-1 (tegafur 120 mg, CDHP 34.8 mg, potassium oxonate 117.6 mg) was administered orally. A regime was established consisting of oral administration of S-1 for 28 days followed by an intermission of 14 days. Complete disappearance was achieved, when 2 courses were given. The lesion showed pigmented macules and an ulcer, 40 × 30 mm in size, with no pain (fig. 2a). A spindle biopsy from the border of the ulcerated lesion was taken for histopathological evaluation. The specimen revealed a massive infiltration of inflammatory cells in the dermis, dilated vessels, and extravessel erythrocytes (fig. 2b). The infiltrated cells were intermingled with plasma cells, lymphocytes and histiocytes (fig. 2c).

However, 2 elastic hard papules, 5 × 5 mm in size, developed on the previously affected lesion after the end of the third course of the S-1 administration (fig. 3a). A biopsied specimen from a papule showed tumor cells that were morphologically irregular in size and shape with large, pleomorphic and hyperchromatic nuclei (fig. 3b, c). Immunohistochemical staining showed positive reaction to GCDFP-15 and negative reaction to mammaglobin, HER2, ER and PgR.

Discussion

The skin lesion was diagnosed as skin metastases from possible occult breast cancer. Occult breast cancer commonly presents lymph node metastasis of the axilla, supraclavicular fossa and infraclavicular fossa as first manifestation [4, 5]. One differential diagnosis is primary carcinoma of an accessory mammary gland. The histopathological specimen is characterized by the presence of sweat glands, sebaceous glands, and connective tissue [6].

Chemotherapy consisting of epirubicin and cyclophosphamide is commonly given as the first-line therapy for metastatic breast cancer. Taxanes, such as paclitaxel, are used in early-stage breast cancer and in first-line therapy for metastatic breast cancer [7]. Treatment of taxane-refractory disease is challenging and newer agents are being developed for clinical application [7]. S-1 is an oral fluoropyrimidine formulation that combines tegafur, CDHP, and potassium oxonate in a molar ratio of 1:0.4:1 [8]. CDHP inhibits an enzyme that metabolites 5-FU, dihydropyrimidine dehydrogenase [9]. Therefore, high 5-FU concentrations are maintained, and S-1 may be effective in patients who do not respond to other fluoropyrimidine agents [9]. Potassium oxonate can reduce gastrointestinal toxicities, which are the main adverse events of 5-FU agents [9]. Single agent S-1 has demonstrated marked activity against a broad array of solid tumors, including breast, gastric, colorectal, cervical, pancreatic, and lung cancers [8]. The strategy for treating metastatic breast cancer is to maintain favorable quality of life and to improve survival [9]. S-1 may be a valuable agent for metastatic and recurrent breast cancer because it shows clinical efficacy and mild toxicity and can be given orally [9]. A randomized controlled trial is currently comparing S-1 with standard chemotherapies, such as taxanes, in women with metastatic or recurrent breast cancer [10]. In the present case, temporal complete disappearance was achieved. We believe that the administration of S-1 temporarily kept the patient in a comfortable condition and improved the period of survival.

In summary, we described the case of a male patient with skin metastases from possible occult breast cancer. S-1 may be a candidate for chemotherapy for skin metastases from breast cancer in males.
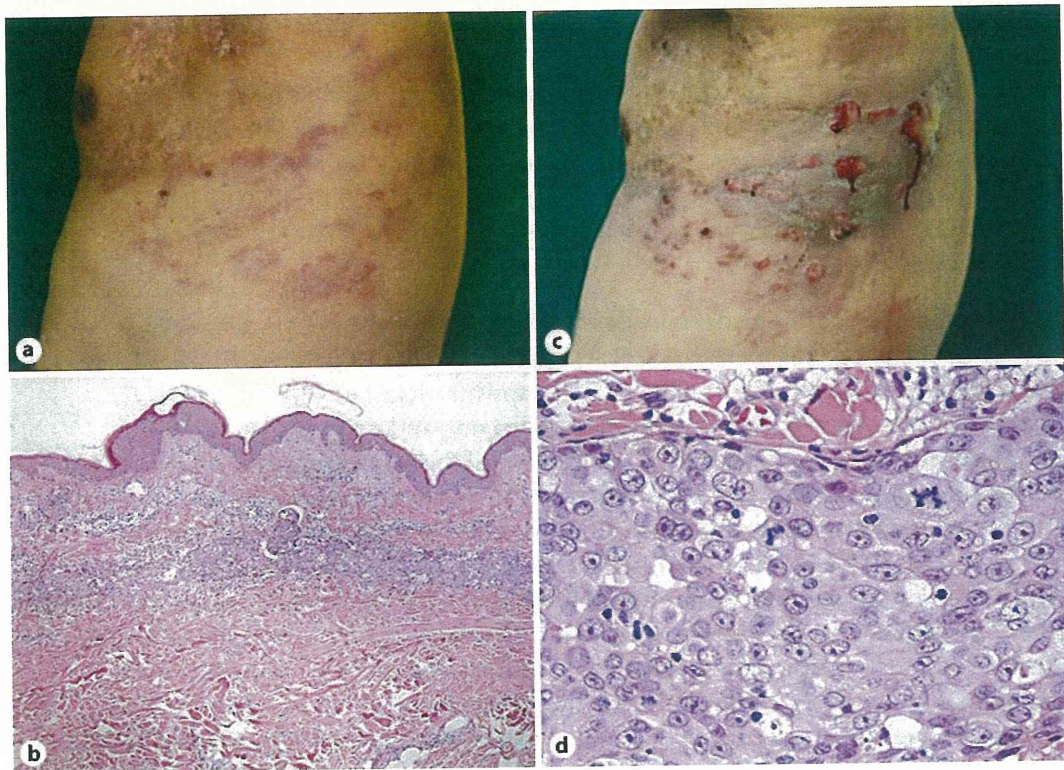
Fig. 1. a Clinical image of erythematous patches and linear macules on the left chest and back. **b, d** A biopsied specimen from an erythematous linear macule. HE, original magnification, ×100 (**b**), ×400 (**d**). **c** Clinical image of a large dark reddish patch with necrotic nodules after 5 courses of resumed chemotherapy with paclitaxel.

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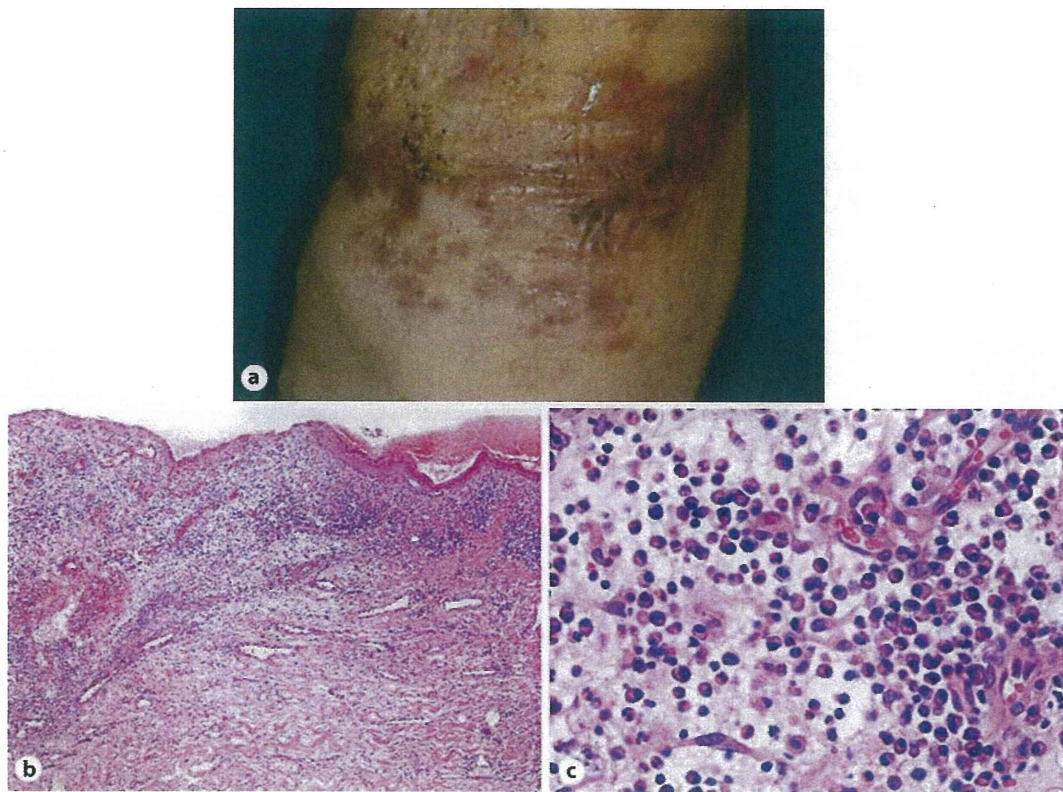
Fig. 2. a Clinical image of complete disappearance of the metastatic skin lesion after 2 courses of S-1 therapy. **b, c** A biopsied specimen from the border of the ulcer. HE, original magnification, ×100 (**b**), ×400 (**c**).
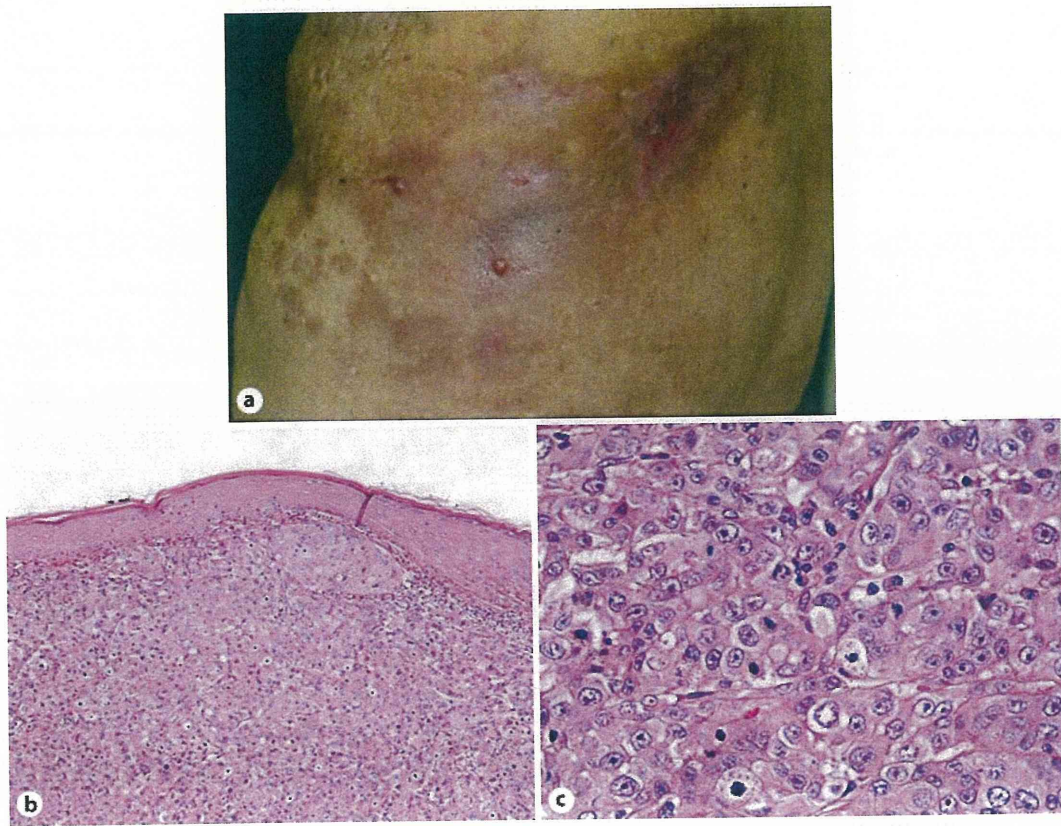
Fig. 3. a Clinical image of 2 elastic hard papules, 5 × 5 mm in size, that recurred after the end of the third course of the S-1 administration. **b**, **c** A biopsied specimen from a papule. HE, original magnification, ×100 (**b**), ×400 (**c**).

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KARGER

特集 進化するがん免疫療法（ワクチン療法，細胞療法，抗体療法）

実地医療と臨床試験 1）がん特異的免疫治療の 進歩と細胞治療の問題点*

西村孝司**

はじめに

　1991年，ベルギーのテリー・ブーン博士らによるがん抗原の発見によって，がん免疫治療の開発研究は飛躍的発展を遂げた．キラーT細胞を標的としたがん抗原ショートペプチドを用いたがんワクチン治療では，がんの縮小効果は弱いが延命効果は証明された．世界の動きは，さらに，ヘルパーT細胞とキラーT細胞の両者を活性化できる次世代ロングペプチドの時代に突入し，がん患者生体内でTh1依存的ながん特異的免疫を誘導できるようになった．これと呼応してがん特異的エフェクターの誘導が容易になり，細胞治療も，lymphokine-activated killer(LAK)細胞や活性化T細胞などを用いた非特異的免疫療法から，がん特異的免疫誘導を目的とする樹状細胞(dendritic cell；DC)，キラーT細胞あるいはヘルパーT細胞を用いたがん特異的免疫細胞治療へと大きな舵が切られている．

　本稿では，がん患者が高額の医療費を払い，自由診療でがん治療を受ける際の一つの道標になることを期待して，これまで研究開発されてきた免疫治療の理論的背景や歴史を述べ，現在，日本において実施されている細胞治療の問題点，さらには今後の展望についても述べる．

がん免疫療法の歴史的背景

　われわれの体をウイルスやがんから守る免疫システムに細胞性免疫と体液性免疫が存在するように，がん免疫療法にも①細胞性免疫の賦活に主眼をおいた能動免疫療法と②抗体を用いた受動免疫療法が存在する．さらに，①のがん免疫療法は大きく2つの方法に大別される．一つは免疫反応を惹起する物質を生体内に投与（接種または摂取）することによって，体内の細胞性免疫を賦活するもので，免疫アジュバント療法，サイトカイン療法，あるいはがん抗原ワクチン療法がある．また，広義で健康食品もこの範疇に該当すると考えられる．もう一つは，免疫反応を担う末梢血リンパ球などを患者から一度体外に取り出し，試験管内で刺激，活性化，さらに増殖させたあとに，再び患者の生体に戻し移入する養子免疫療法がある．30年前に発見されたIL-2で活性化されたリンパ球(LAK)を用いるLAK療法が代表的な免疫細胞治療である[1)2)]．さらに，これまでNK細胞，NKT細胞，$\gamma\delta$T細胞，cytotoxic T lymphocytes(CTL)，helper T(Th)細胞の移入療法などが順次新しい免疫細胞療法として登場してきている（図1）．がん特異的免疫治療が可能であると考えられるようになったのは，1991年，ベルギーのテリー・ブーン博士の研究グルー
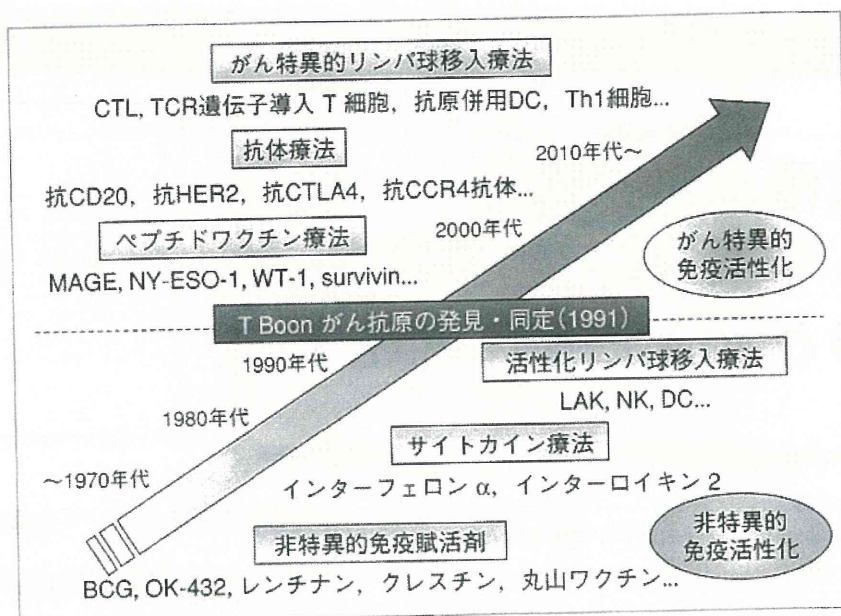
図1　がん免疫療法の歴史

プが，がん細胞に特異的に発現しているがん抗原を発見・同定[3]してからである．がん抗原の発見当初は，がん細胞を直接攻撃するCD8^{+}キラーT細胞を標的としたMHCクラスⅠ拘束性のペプチドワクチン療法が盛んに開発され，数多くの臨床試験が実施されてきた．アミノ酸8〜10個からなるクラスⅠがん抗原ペプチドを用いたがんワクチン治療は，がん特異的キラーT細胞の誘導能やがん縮小効果も弱いとRosenbergら[4]が発表したが，最近わが国で，化学療法との併用によって延命効果があることが認められた[5)6]．しかし，当初期待されたがん縮小効果は弱く，最近では，キラーT細胞とヘルパーT細胞の両者を活性化できるヘルパーエピトープとキラーエピトープの両者を併せ持った天然がん抗原ペプチド[7]や両者を人工的に結合させたhelper/killer-hybrid epitope long peptide（H/K-HELP）[8]も開発され，有効性が確認され始めている．世界のがんペプチド研究の舞台は，キラーT細胞のみを標的としたショートペプチドからヘルパーT細胞とキラーT細胞の両者を標的としたロングペプチドに移行し始めているのが現状である．

がん特異的な細胞治療の現状

　がんの細胞治療とは，がん細胞を攻撃する機能を持つ免疫細胞（リンパ球）を体外に取り出し，

GMPレベルの細胞培養施設（CPC）でサイトカイン，免疫アジュバントを用い，場合によっては自己がん組織あるいは人工がん抗原蛋白質あるいはペプチドの存在下で大量に数を増やし，加工・処理することにより高機能を付与した上で，再びがん患者の体内に戻すがん免疫療法である（図2）．がん抗原特異性のないLAK療法，αβT細胞療法，γδT細胞療法，NK細胞療法，NKT細胞療法などが，現在わが国でも実施されているが，本稿では，今後治療効果が期待される，がん特異的免疫細胞治療であるCTL療法，樹状細胞療法，Th1細胞治療について述べる．

1．CTL療法

　がん患者より回収したTリンパ球を培養・活性化する際，患者自身のがん細胞を同時に添加することで，がん細胞特異的に殺傷能力を持ったCTLを活性化，増殖させ，体内に戻す治療法として考案された．手術や胸水・腹水サンプルなど，患者自身のがん細胞が提供されうる場合に適応が可能で，一般に正常細胞になく，がん細胞だけにあるがん抗原特異的なCTLをいかにして効率よく増殖させるかという研究が世界中でなされている．しかしながら，体外で行うCTLの誘導培養は作業が煩雑な上，成功率も決して高いものではなく，臨床応用するためには多くの課題が存在している．がん抗原が発見されてから
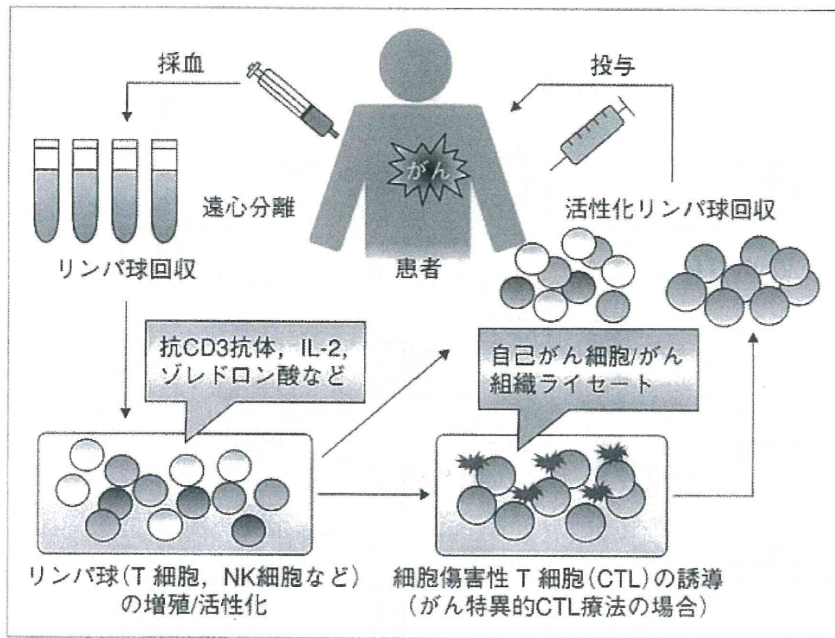
図2　活性化リンパ球移入療法

は，がん抗原ペプチド刺激でCTLを増殖させることも可能になった．しかし，CTLはIL-2を多く産生することができず，生体内寿命が短いことが問題である．最近は，この問題を克服するために，生体内での生存が長期期待できるエフェクターメモリーCTL[9]やT細胞レセプター（TCR）やキメリック抗原レセプター（CAR）遺伝子導入CTLを用いた細胞治療が行われている[10)11]．特にCAR遺伝子導入CTLは良好な成果をおさめており，多くの腫瘍縮小例も出ている[12]．

2．樹状細胞療法

　樹状細胞は体内で損傷を受けたがん細胞を貪食し，MHCクラスⅠあるいはクラスⅡにがん抗原を提示し，最終エフェクターであるT細胞に抗原を提示する働きを担う．この樹状細胞を用いて特異的がん免疫の活性化を期待するのが樹状細胞ワクチン療法である．樹状細胞療法には3つの方法があり，1つは患者のがん組織・がん細胞を利用した自己がん組織樹状細胞療法，2つめは人工抗原を利用した人工抗原樹状細胞療法，3つめは自己がん組織・がん細胞や有望な人工抗原がない人に対して樹状細胞をがんに直接注入する局所樹状細胞療法である．手術でがん組織が採取できる場合には自己がん組織樹状細胞療法が期待される．自己のがん組織が採取で

きないが，がん抗原の発現がチェックでき，使用可能ながん抗原蛋白質あるいはペプチドが入手可能である場合には，がん抗原蛋白あるいはがん抗原ペプチドをパルスした樹状細胞を用いた樹状細胞療法が施行できる．局所樹状細胞療法は上の2つの治療のような準備や条件の必要はなく，がんの場所が樹状細胞を直接注入できるような所に存在するかどうかが治療実施の条件となる．

　樹状細胞は，培養中ではほとんど増殖しない．そのため，細胞の調製・加工にはアフェレーシスなどによる大量のリンパ球の採取が必要となる．回収した単球や未熟な樹状細胞を体外においてサイトカインおよび免疫アジュバントを用いて分化誘導し，抗原提示能力が非常に強い成熟細胞を作出したあと，さまざまながん細胞やがん抗原蛋白質あるいはペプチドを共存させ，大量のがん抗原を樹状細胞内にパルスする．このがん抗原パルス樹状細胞を皮下注射し，体内でがん抗原特異的なヘルパーT細胞やキラーT細胞の活性化を目指す（図3）．これまでベルギーのテリー・ブーン博士のグループは，メラノーマのがん抗原ペプチドをのせた樹状細胞を[13]，スイスのF・ネッスル博士らはがん細胞溶解液をのせた樹状細胞を[14]，皮膚がん患者に注射し有望な治療成績を報告してい
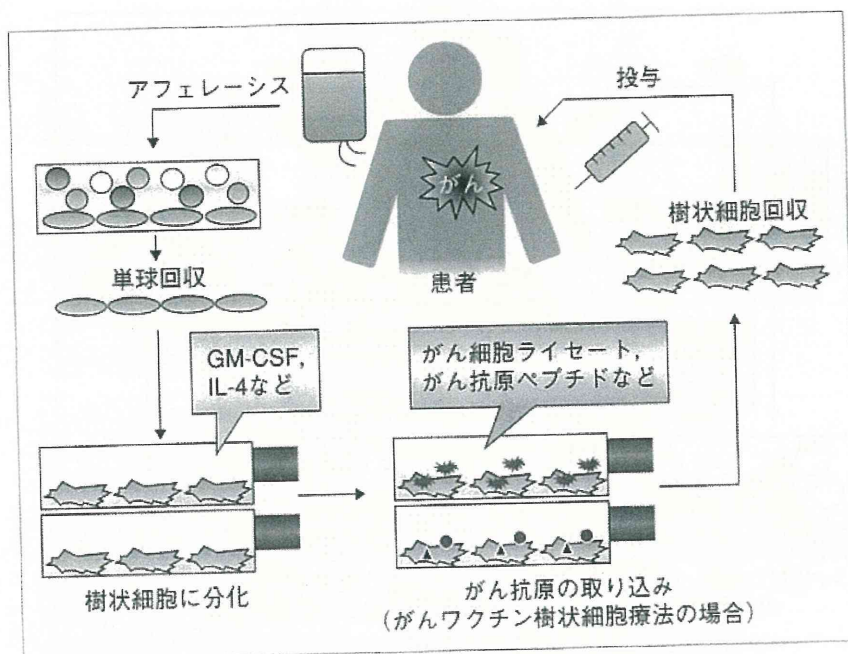
図3　樹状細胞移入療法

る．最近，米国デンドリオン社の開発した樹状細胞治療がFood and Drug Administration（FDA）で承認されたことに端を発して樹状細胞治療はさらに発展する可能性がある．われわれもバイオイミュランスやテラとの連携でH/K-HELPの樹状細胞療法への応用を考えている．

3．がん特異的Th1細胞移入療法

　免疫バランスは主に2種類のヘルパーT細胞（Th1/Th2細胞）の相互調節によって制御されている．がんの免疫応答はTh1型免疫を活性化することによってがん特異的キラーT細胞（Tc1またはCTL）を含む細胞性免疫が活性化されることが重要である．このことはがん特異的キラーT細胞を増殖・維持するためには，IL-2を含むサイトカインなどを豊富に産生するTh1細胞からのヘルプが必要であることを示唆している．さらに，①がん細胞がクラスII分子を発現している場合には，がん特異的Th1の移入によってがんを拒絶できること[15]，②がんがクラスIIを発現していない場合でも，がん特異的Th1細胞とがん抗原蛋白あるいはクラスIペプチドおよびクラスIIペプチドを組み合わせた治療法によってがんの増殖制御が可能であること[16]，③Th1型免疫を強力に活性化できるCpG-ODNや溶連菌OK-432をアジュバントとし，がん抗原蛋白やクラスIおよびクラスIIペプチドとともにリポソームに封入した混合物を所属リンパ節近傍に接種することによって，治療中に十分量のがん特異的キラーT細胞が担がん生体内で誘導され，がんを拒絶できること[17)18]を証明しており，Th1細胞移入療法が次世代型の新しいがん免疫治療になることが期待される．このTh1細胞治療の特筆すべき点は，がんや所属リンパ節にがんの増大とともに誘導されるTregの増加，蓄積がIFN-γ依存的に抑制できる点である[19]．また，Th1微小環境でTc17なども抗腫瘍エフェクターに変換させる利点もある．すでに，われわれは放射線療法や化学療法とTh1細胞治療との併用療法がより有効であることも確認している[20]．

　以上の科学的根拠に基づいて，MAGE-A4抗原を発現する頭頸部がん，乳がん，非小細胞肺がんまたは消化器がんを含むすべての難治性がん種の進行・再発がん症例に対して，MAGE-A4あるいはsurvivin特異的Th1細胞を誘導し，可能であればがん局所に，あるいは困難であれば近傍皮下に投与する治療法を考案した．移入されたTh1細胞はヘルパーペプチドによる刺激，あるいは体内の抗原提示細胞とのがん抗原ペプチドを介した相互作用によりタイプIサイトカインを産生することでがん患者生体内にがん抗原特異