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Specific Tumoricidal Activity of a Secreted Proapoptotic Protein Consisting of HER2 Antibody and Constitutively Active Caspase-3[1]

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ABSTRACT

In this study, a novel approach to antitumor therapy was devised by generating a chimeric tumor-targeted killer protein, referred to as immunocasp-3, that comprises a single-chain anti-erbB2/HER2 antibody with a NH_2-terminal signal sequence, a *Pseudomonas* exotoxin A translocation domain, and a constitutively active caspase-3 molecule. In principle, cells transfected with the immunocasp-3 gene would express and secrete the chimeric protein, which then binds to HER2-overexpressing tumor cells. Subsequent cleavage of the constitutively active capase-3 domain from the immunocasp-3 molecule and its release from internalized vesicles would lead to apoptotic tumor cell death. To test this strategy, we transduced human lymphoma Jurkat cells with a chimeric immunocasp-3 gene expression vector and showed that they not only expressed and secreted the fusion protein but also selectively killed tumor cells overexpressing HER2 *in vitro*. i.v. injection of the transduced Jurkat cells led to tumor regression in a mouse xenograft model because of continuous secretion of immunocasp-3 by the transduced cells. The growth of HER2-positive tumor cells in this model was inhibited by i.m. as well as intratumor injection of immunocasp-3 expression plasmid DNA, indicating that the immunocasp-3 molecules secreted by transfected cells have systematic antitumor activity. We conclude that the immunocasp-3 molecule, combining the properties of a tumor-specific antibody with the proapoptotic activity of a caspase, has potent and selective antitumor activity, either as cell-based therapy or as a DNA vaccine. These findings provide a compelling rationale for therapeutic protocols designed for erbB2/HER2-positive tumors.

INTRODUCTION

Mammalian cells have evolved elaborate mechanisms of programmed cell death (apoptosis), which is characterized by the activation of caspases and consequent widespread biochemical and morphological perturbations resulting in cell death (1–6). Among the critical cysteine protease family members, caspase-3 plays an essential role in the cleavage of diverse cellular proteins (5, 7, 8). Caspase-3 exists as a proenzyme in the cell, consisting of an NH_2-terminal prodomain, a large subunit, and a small subunit. Upon activation by upstream stimuli, the precursor molecule is cleaved at specific CPSs[3] (consisting of several amino acid residues, including -Ile-Glu-Thr-Asp-) between the domains, resulting in the removal of the prodomain and the reassociation of the large and small subunits to form a heterodimer (5, 9–11). Srinivasula *et al.* (12) reported the constitutive activity of a contiguous caspase-3 precursor (C-cp3) in which the small subunit was placed before the large one (reverse of the normal order).

Despite limited clinical success, tumor-targeted gene therapy remains a promising approach to the treatment of malignant diseases (13–16). Tumor cells are distinguished by a class of specific antigens, including erbB2/HER2, a growth factor receptor-like oncoprotein that is widely overexpressed on the surface of tumor cells, especially those of breast, ovary, and stomach carcinoma (17–21). We describe here the generation of a caspase-3-based fusion protein, termed immunocasp-3, consisting of an erbB2/HER2 single-chain antibody (e23sFv; Refs. 18, 22–24), a translocation domain of PE (25–28), and a C-cp3 molecule (12). Because of the high affinity of e23sFv for HER2, this chimeric protein was anticipated to bind selectively to HER2-overexpressing cancer cells, followed by its internalization and lysosomal cleavage (29). The resulting COOH-terminal peptide, containing C-cp3 and a portion of PE domain II, would then be released into the cytosol, where it would induce apoptotic cell death (26, 27). To test these predictions, we genetically modified human lymphoma Jurkat cells to secrete immunocasp-3 as the result of a NH_2-terminal signal sequence fused to the single-chain antibody (29, 30) and evaluated their *in vitro* and *in vivo* antitumor activities.

MATERIALS AND METHODS

Plasmid Construction. The human caspase-3 gene was amplified by PCR from cDNA derived from human lymphoma Jurkat cells lines. A C-cp3 gene was generated by switching the order of coding sequences for the large and small subunits, using a PCR-based gene splicing by overlap extension method (31). The genes encoding anti-HER2 single-chain antibody (e23sFv) and PE were generated in our previous study (29). To form P-cp3 genes, we infused the portion of the PE gene corresponding to amino acids 280–412 or 280–364 in frame with the 5′-end of the C-cp3 gene by gene splicing by overlap extension, creating an upstream *Eco*RI site and a downstream *Sal*I site. Four P-cp3s with a CPS were constructed by adding a sequence encoding 10 amino acids (Ser-Glu-Ser-Asp-Cys-Gly-Ile-Glu-Thr-Asp) between fragments encoding PE peptide and C-cp3. These desired genes were digested with the above restriction endonucleases and ligated to the corresponding multiple cloning site of pIR (pIRES2-EGFP, a Clontech product), which permitted the independent expression of the protein of interest and the enhanced green fluorescent protein from a single bicistronic mRNA. Generation of the immunocasp-3 gene involved a sequential fusion of the genes of a signal peptide (Met-Lys-His-Leu-Trp-Phe-Phe-Leu-Leu-Leu-Val-Ala-Ala-Pro-Arg-Trp-Val-Leu-Ser), e23sFv, the PE translocation domain, and C-cp3. The gene was then cloned into the *Hin*dIII/*Xba*I site of pCMV vector. All vector sequences were confirmed by DNA sequencing.

Transient Transfection of HeLa, Western Blotting, and MTT Assay. Human mammary carcinoma HeLa cells were cultivated in DMEM containing 10% FCS and transfected with liposome-encapsulated pIR-P-cp3 or pIR-C-cp3. Cell lysates were prepared, separated by SDS-PAGE, and analyzed by Western blotting, using a caspase-3 antibody. For the MTT assay, HeLa cells transfected with P-cp3 or C-cp3 genes were cultivated in 96-well plates for 24–96 h, incubated with 20 μl of 1.5 mg/ml MTT per well for 4 h. A total of 150 μl of DMSO was added, and the $A_{490\ nm}$ values were assayed by an ELISA reader.

Generation of P-cp3/C-cp3-inducible HeLa Cells. A commercial inducible mammalian expression system Complete Control (Stratagene) was used to generate inducible HeLa cells. First, P-cp3 and C-cp3 genes were subcloned into the *Xho*I/*Sal*I site of the pEGSH vector. HeLa cells were transfected with

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[3] The abbreviations used are: CPS, caspase-processing site; C-cp3, constitutively active caspase-3; FACS, fluorescence-activated cell sorting; i.t., intratumoral; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P-cp3, a fusion protein consisting of PE aa 280–412 (or 364) and C-cp3; PE, *Pseudomonas* exotoxin A; WT, wild-type.

a pERV3 plasmid that expressed a chimeric ecdysone receptor, VgEcR, and the selected clones were transiently transfected with pEGSH-luciferase. In the presence of ecdysone or its analogue, VgEcR became activated and bound to a specific cis element on pEGSH to initiate transcription of the downstream gene. Cellular luciferase activities were then assayed with a Promega kit before and after addition of 5 μM ponasterone A, an ecdysone analogue. The cell clone with the lowest background expression and highest inducing folds was chosen for additional transfection with pEGSH-P-cp3, pEGSH-C-cp3, and pEGSH-WT caspase-3 to establish gene inducible HeLa cells.

Caspase-3 Activity Assay. Cellular caspase-3 activities were assayed with a Boehringer-Mannheim kit. Briefly, P-cp3, C-cp3, or WT caspase-3-inducible HeLa cells were lysed before or 24 h after induction with 5 μM ponasterone A, and the supernatants were prepared for centrifugation. A microwell test plate was coated and blocked with designated buffer. Samples, as well as positive or negative control reagents, were added into wells and incubated at 37°C for 1 h, followed by the addition of the substrate acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethyl-coumarin (Ac-DEVD-AFC). Free AFC was determined fluorometrically at 505 nm, and caspase-3 activities were quantified by a calibration curve, which was created by serial dilutions of AFC substrates. Each assay was performed with triplicate wells.

Generation of Immunocasp-3-secreting Jurkat Cells. Jurkat cells were transfected with pCMV-immunocasp-3 or control vector, followed by selection with G418 (600 μg/ml). The resulting cells were cultivated in RPMI 1640 containing 10% FCS, and the expression of immunocasp-3 was determined by reverse transcriptase-PCR. The expression and secretion of the proteins were examined by Western blotting using a caspase-3 antibody.

Examination of *in Vitro* Antitumor Activity of Immunocasp-3-secreting Lymphocytes. Human mammary carcinoma SKBR-3, ovary carcinoma SKOV-3, cervical carcinoma HeLa, and umbilical vein endothelium-derived ECV-304 cells were first analyzed by FACS for the expression of erbB2/HER2. Cells were stained with rabbit antihuman erbB2/HER2 antibody (Oncogene) at 37°C for 1 h, followed by incubation with mouse antirabbit IgG-FITC at 37°C for 30 min. Cells positive for erbB2/HER2 were sorted on a FACSVantage (Becton Dickinson Systems).

HER2-overexpressing cancer cells (SKBR-3 and SKOV-3) and control cells (HeLa and ECV-304) were cocultivated with immunocasp-3 gene-modified Jurkat cells (with a 1:3 ratio of tumor cells to secretor cells) on 96-well plates for various times. Tumor cells were counted, and the percentages of cell killing were calculated at these times. Meanwhile, tumor cells were harvested after cocultivation with immunocasp-3-secreting Jurkat cells for 72 h, and the genomic DNA were extracted and analyzed using an apoptotic DNA ladder kit (Boehringer Mannheim).

Assessment of *in Vivo* Antitumor Activity of Immunocasp-3. An SKBR-3 xenograft mouse model was established by s.c. injection of 2×10^6 cells in the right inguina. One group of mice received six doses of 5 μg of pCMV-immunocasp-3 mixed with liposome, twice a week i.t. or i.m. in the right posterior limb. Another group of mice received three weekly i.v. injections of 10^6 Jurkat-immunocasp-3 cells. Control mice were injected with empty pCMV vector or unmodified Jurkat cells. Each treatment was performed on five mice. Tumor volumes were measured and mouse survival was recorded for additional analysis.

For histological analysis, tumors from the above-treated mice were fixed in either Bouin's fixative or 10% neutral-buffered formalin, and then processed in paraffin, sectioned at 4 μm, and subjected to indirect immunofluorescence examination. Briefly, endogenous peroxidase was quenched with 3% H_2O_2 in distilled water, and sections were blocked for 2 h at room temperature in PBS containing 0.2% Triton X-100 and normal bovine serum. They were then successively incubated with rabbit anti-active caspase-3 antibody overnight at 4°C and biotinylated goat antirabbit IgG at 37°C for 1 h in PBS supplemented with 0.2% Triton X-100. The secondary antibody was detected with streptavidin-biotin complex conjugated to Texas Red.

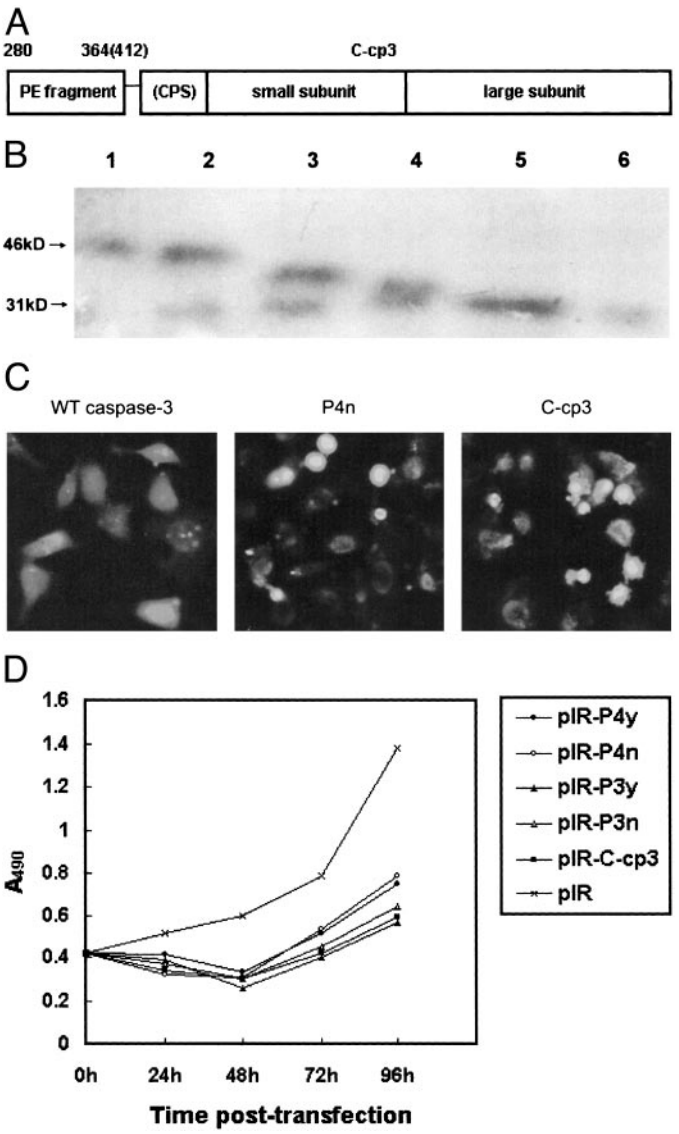

Fig. 1. Cytotoxicity of P-cp3 is comparable with that of C-cp3. *A*, P-cp3 consists of PE aa 280–412/364 and C-cp3, with or without a CPS between them; CPS, caspase processing site (10 amino acid residues, including -Ile-Glu-Thr-Asp-). *B*, Western blotting of cell lysates using a caspase-3 antibody at 12 h after transfection: *Lane 1*, P4y; *Lane 2*, P4n; *Lane 3*, P3y; *Lane 4*, P3n; *Lane 5*, C-cp3; *Lane 6*, control. P4y, P4n, P3y, P3n represent the P-cp3 constructs with different NH_2-terminal peptides, as follows P4y, PE aa 280–412-CPS-C-cp3; P4n, PE aa 280–412-C-cp3; P3y, PE aa 280–364-CPS-C-cp3; P3n, PE aa 280–364-C-cp3. *C*, fluorescence microscopy of HeLa cells at 24 h after transfection with the indicated genes. *D*, curves of MTT assays of HeLa cells performed at 24–96 h after transfection with different gene constructs.

RESULTS

NH_2-Terminal Fusion of a PE-derived Short Sequence Does not Abrogate the Activity of C-cp3. PE is a single-chain toxin consisting of 613 amino acids and three major domains, termed Ia (amino acid (aa) 1–252), II (aa 253–364), and III (aa 400–613), which are responsible for the binding of targeted cells, translocation to the cytosol, and ADP ribosylation of protein elongation factor-2 to induce cell death, respectively. As argued by Prior *et al.* (26), aa 253–412 also appears necessary for a complete translocation function of the cytotoxic protein. Domain Ib (aa 365–399) is a minor domain with undefined function. After PE enters a cell, a proteolytic cleavage occurs near Arg^{279}, generating a COOH-terminal fragment, which in turn translocates to the cytosol and kills the cell (25, 27). Immunotoxins have been made by substituting targeting molecules such as antibodies for domain Ia without interfering with the functions of other domains (27, 28). In our strategy, the internalized immunocasp-3 molecule would release into the cytosol a COOH-terminal peptide, P-Cp3, consisting of a portion of PE (aa 280–364 or aa 280–412) and C-cp3. Experiments were therefore needed to deter-

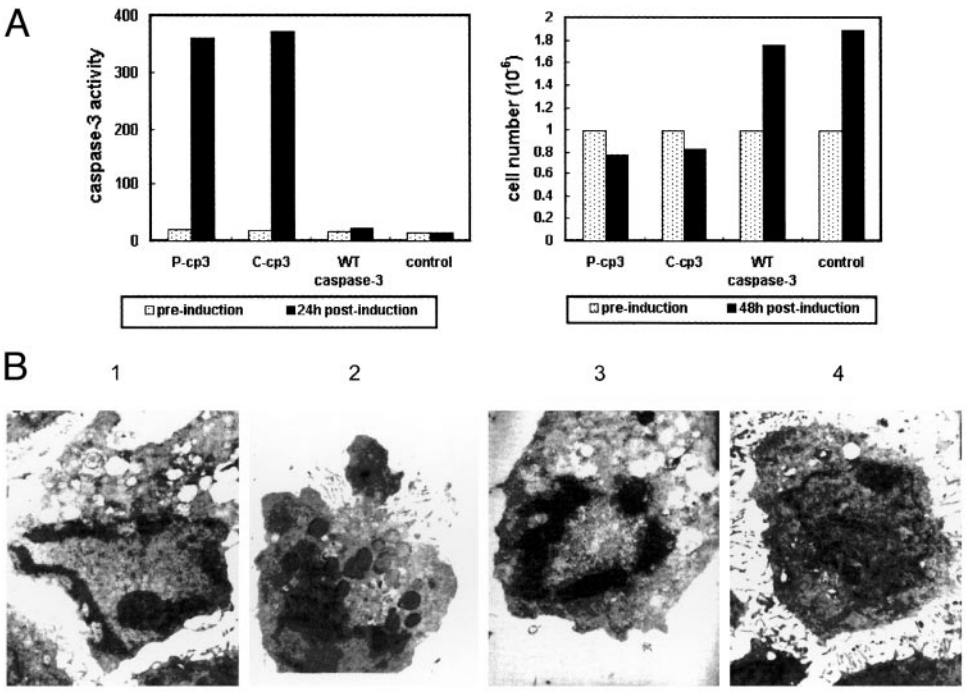

Fig. 2. Treatment of P-cp3/C-cp3-inducible HeLa cells with ecdysone analogue causes apoptosis. A Complete Control (Stratagene) system was used to generate P-cp-3 and C-cp-3-inducible HeLa cells. *A*, intracellular caspase-3 activities, and numbers of HeLa cells pre- and postinduction of the indicated genes. A total of 2×10^6 cells was lysed 24 h after addition of 5 μM ponasterone A; caspase-3 activities were assayed with a Boehringer Mannheim kit based on the cleavage of DEVD-AFC to release fluorescence-free AFC. Cells were counted before and 48 h after addition of ponasterone A. *B*, electronic microscopy of HeLa 48 h after induction of caspase-3 gene constructs with 5 μM ponasterone A. *1*, P-cp3 (×6000); *2*, P-cp3 (×5000); *3*, C-cp3 (×6000); *4*, WT caspase-3 (×5000).

mine whether the PE-derived sequence interfered with the activity of C-cp3.

Because the boundaries of the functional PE translocation domain were not clearly defined, four types of the P-cp3 gene were generated by fusing fragments encoding PE aa 280–412 or aa 280–364 to the C-cp3 gene, with or without the CPS between them (Refs. 11, 25, 26; Fig. 1*A*). These P-cp3 genes were transiently expressed in human cervical carcinoma HeLa cells (Fig. 1*B*), and the cytotoxic effects were evaluated. Cells expressing each of the four P-cp3 gene types and C-cp3 from a green fluorescent protein-expressing vector exhibited fatal morphological changes as well as comparably inhibited cell growth as demonstrated by MTT assays (Fig. 1, *C* and *D*). The cell killing efficiency of P-cp3 constructs containing PE aa 280–412 was similar to that of constructs containing PE aa 280–364, and the presence of CPS did not promote caspase-3 activity (Fig. 1*D*). The cleavage of poly(ADP-ribose) polymerase was detected in cells transfected with the P-cp3 variants and C-cp3 but not in vector-transfected cells (data not shown). The P-cp3 construct containing PE aa 280–412 but no CPS was chosen for additional study.

The proapoptotic activities of P-cp3, C-cp3, and WT caspase-3 were investigated in HeLa cells inducibly expressing these molecules. As shown in Fig. 2*A*, the expression of P-cp3 or C-cp3 resulted in 70–80-fold increases of cellular caspase-3 activity upon induction with ponasterone, whereas the increase of caspase-3 activity in WT caspase-3-inducible cells was negligible. The expression of P-cp3 or C-cp3 also accounted for 59.3 and 56.1% inhibition of cell proliferation, respectively, in contrast to 6.9% in WT caspase-3-inducible cells (Fig. 2*A*). As observed by electronic microscopy, the expression of P-cp3 or C-cp3 produced typical apoptotic changes, including chromatin condensation and its margination at the nuclear periphery, cellular shrinkage and blebbing, and formation of so-called apoptotic bodies (Fig. 2*B*). Thus, C-cp3 and P-cp3 containing a PE-derived short peptide induced a comparable caspase-3-like activity.

HER2-overexpressing Tumor Cells Are Selectively Killed *in Vitro* by Immunocasp-3 Gene-modified Jurkat Cells. The immunocasp-3 gene was constructed by fusing the genes for the anti-HER2 single-chain antibody (e23scFv) and the PE translocation domain (PE aa 253–412) in-frame with the 5′-end of C-cp3 gene. The resulting fusion gene also contained a coding sequence for NH_2-terminal signal peptide (Refs. 29, 30; Fig. 3*A*). Jurkat cells were then transduced with immunocasp-3, with the intent of inducing them to secrete the targeted protein to kill HER2-overexpressing tumor cells. As expected, the immunocasp-3-modified Jurkat cells remained viable and showed growth and proliferation properties that were similar to those of unmodified cells (data not shown), suggesting low levels of toxicity from expression of the chimeric protein. The presence of the immunocasp-3 gene in modified Jurkat cells was confirmed by reverse transcriptase-PCR (data not shown), and the secreted protein was detected in culture medium by Western blotting (Fig. 3*B*) and ELISA (data not shown).

The genetically modified Jurkat cells were then cocultivated *in vitro* with HER2-positive human tumor cells, SKBR-3 and SKOV-3, and control cells expressing low or undetectable levels of HER2, HeLa (17–22) and ECV-304 (Fig. 3*C*). The ratio of Jurkat:target cells was adjusted to 3:1, based on pilot experiments designed to identify the optimal ratio for cell killing. As shown in Fig. 3*D*, significant numbers of SKBR-3 and SKOV-3 cells, but not HeLa or ECV-304 cells, were killed by immunocasp-3-secreting cells. More than 70% of the cells had died by 3 days of coculture, and additional killing of HER2-overexpressing tumor cells was seen after longer times. Clear DNA ladders were apparent by electrophoresis of genomic DNA isolated from cocultivated SKBR-3 or SKOV-3 cells, suggesting the occurrence of apoptotic cell death (Fig. 3*E*).

HER2-overexpressing Tumors Are Strongly Suppressed *in Vivo* by Injection of Immunocasp-3-expressing Plasmids or Immunocasp-3-modified Jurkat Cells. We next assessed the *in vivo* antitumor activity of immunocasp-3 in a murine xenograft model. SKBR-3 cells were inoculated s.c. into nude mice to form inguinal solid tumors, followed by either administration of liposome-encapsulated pCMV-immunocasp-3 twice a week for 3 weeks or three weekly i.v. injection of 10^6 immunocasp-3-gene-transduced Jurkat cells. i.t. or i.m. administration of immunocasp-3 genes led to potent inhibition of tumor growth and prolonged mouse survival, presumably through modification of tumor or muscle cells to secrete tumor-targeted killer

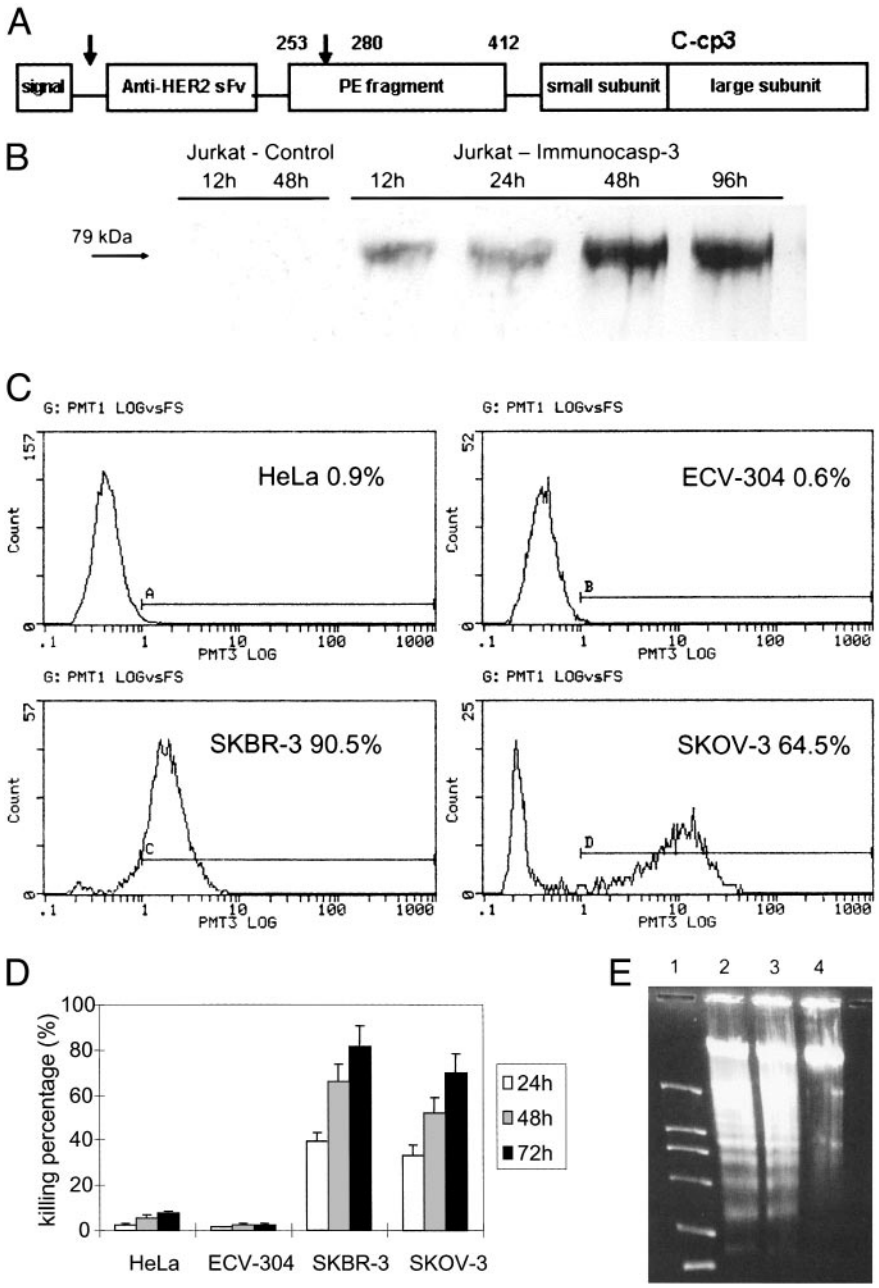

Fig. 3. HER2-overexpressing tumor cells are killed by immunocasp-3 gene-modified Jurkat cells. *A*, schematic diagram of immunocasp-3 comprising an anti-HER2 single-chain antibody, PE translocation domain (PE aa 253–412) and C-cp3. *B*, detection of immunocasp-3 protein secreted by genetically modified Jurkat cells. The clones of genetically modified Jurkat cells were established by transfection of pCMV-immunocasp-3 and subsequent selection with G418. The media of genetically modified or control Jurkat cells were prepared after 12–96 h cultivation of the initially inoculated 2×10^5 cells. The media were enriched and immunoprecipitated with a caspase-3 antibody, the resulting precipitates subjected to SDS-PAGE and Western blotting using the same antibody. *C*, FACS analysis of HeLa, ECV-304, SKBR-3, and SKOV-3 cells with erbB2/HER2 antibody. Cells were stained with rabbit antihuman erbB2/HER2 antibody and FITC-conjugated mouse antirabbit IgG, whereas erbB2/HER2-positive cells were sorted on a FACSVantage. *D*, significant numbers of tumor HER2-overexpressing cells were killed *in vitro* by immunocasp-3 gene-modified Jurkat cells. HER2-overexpressing cancer cells (SKBR-3 and SKOV-3) and control cells (HeLa and ECV-304) were cocultivated with Jurkat-immunocasp-3 (at a ratio of 1:3) on 96-well plates for various times. Tumor cells were counted, and the percentages of dead or dying cells were calculated. *E*, tumor cells were harvested after cocultivation with modified Jurkat cells for 72 h. Genomic DNA was extracted with a DNA ladder kit and separated on 1% agarose gel. *Lane 1*, DNA markers (2000, 1000, 750, 500, 250, and 100 bp); *Lane 2*, SKBR-3; *Lane 3*, SKOV-3; *Lane 4*, HeLa.

protein. Compared with mice that received i.t. injections of pCMV-immunocasp-3, those i.m. treated with equal amounts of plasmids in the right posterior limb had prolonged survival times, presumably because of the longer survival of genetically modified muscle cells and the resultant continuous secretion of immunocasp-3. Meanwhile, i.v. injection of immunocasp-3 gene-transduced Jurkat cells (Jurkat-immunocasp-3) was associated with a remarkable decrease in tumor volume and extremely prolonged mouse survival times, suggesting that this strategy of gene administration yields more effective and sustained antitumor activities in SKBR-3 xenografts (Fig. 4, *A* and *B*). This interpretation is supported by the results of indirect immunofluorescence studies with an active caspase-3 antibody. Caspase-3 activity was detectable in tumor tissues until 3 days after i.t. or i.m. injection of pCMV-immunocasp-3, whereas the caspase-3 activity persisted for at least a week after treatment with immunocasp-3 gene-transduced Jurkat cells (Fig. 4*C*).

DISCUSSION

In the study reported here, we generated a novel antitumor protein, immunocasp-3, which consists of a single-chain antibody against erbB2/HER2, an oncoprotein widely overexpressed on the surfaces of various tumor cells, the translocation domain of PE, and a C-cp3 molecule. Immunocasp-3-secreting lymphocytes were generated by transducing human lymphoma Jurkat cells with the gene encoding this fusion protein. When secreted by the genetically modified cells, the chimeric protein bound to HER2-overexpressing tumor cells, internalized and released a COOH-terminal fragment, which consequently translocates to the cytosol and induces the cell to apoptosis. Lysosomal cleavage of immunocasp-3 between PE amino acids Arg^{279} and Gly^{280} generated a fusion protein consisting of PE aa 280–412 and C-cp3 (26, 28), designated P-cp3, the proapoptotic activity of which was proved by sufficient results. Tumor cells overexpressing HER2,

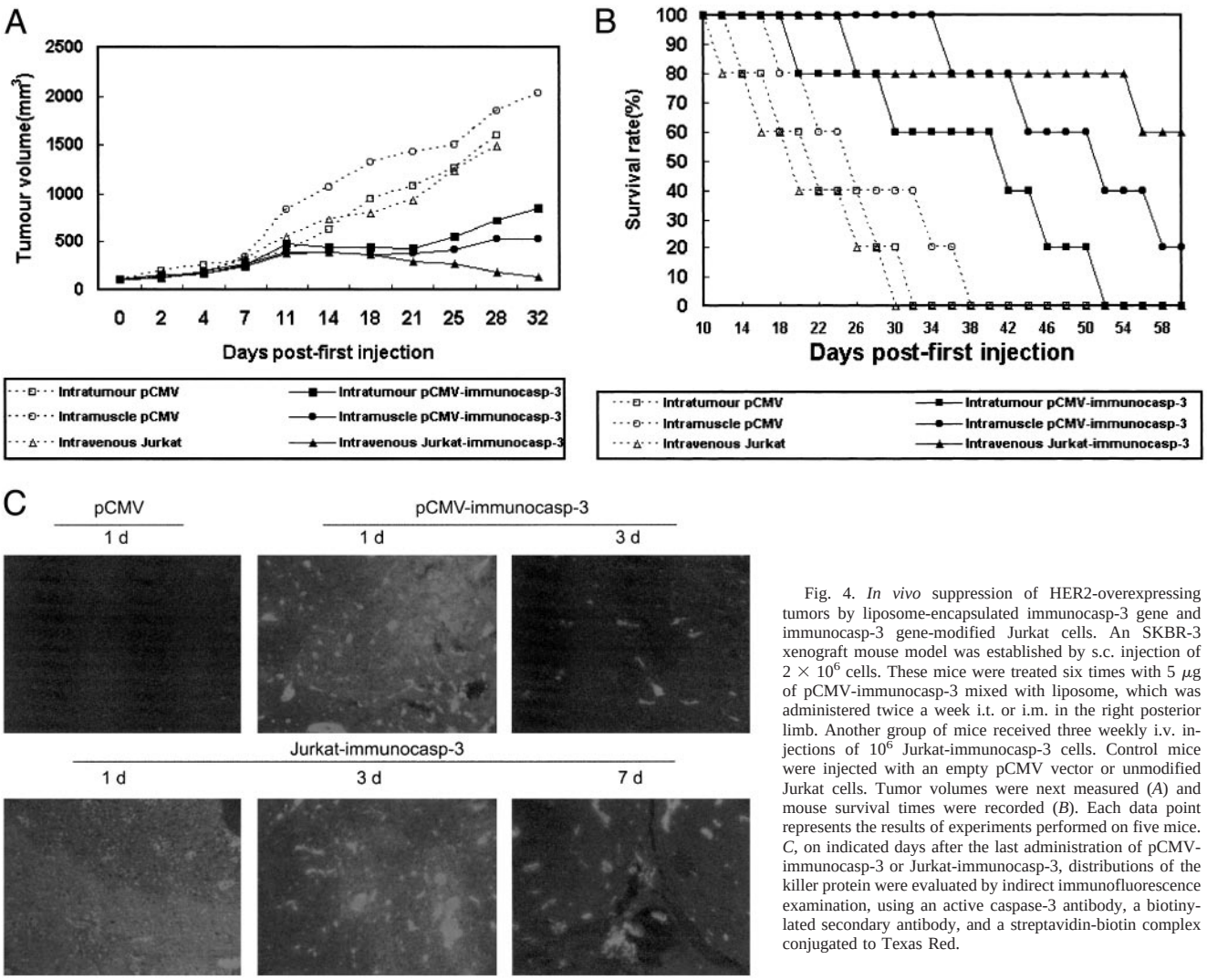

Fig. 4. *In vivo* suppression of HER2-overexpressing tumors by liposome-encapsulated immunocasp-3 gene and immunocasp-3 gene-modified Jurkat cells. An SKBR-3 xenograft mouse model was established by s.c. injection of 2×10^6 cells. These mice were treated six times with 5 μg of pCMV-immunocasp-3 mixed with liposome, which was administered twice a week i.t. or i.m. in the right posterior limb. Another group of mice received three weekly i.v. injections of 10^6 Jurkat-immunocasp-3 cells. Control mice were injected with an empty pCMV vector or unmodified Jurkat cells. Tumor volumes were next measured (*A*) and mouse survival times were recorded (*B*). Each data point represents the results of experiments performed on five mice. *C*, on indicated days after the last administration of pCMV-immunocasp-3 or Jurkat-immunocasp-3, distributions of the killer protein were evaluated by indirect immunofluorescence examination, using an active caspase-3 antibody, a biotinylated secondary antibody, and a streptavidin-biotin complex conjugated to Texas Red.

but not those with normal or undetectable HER2 levels, were killed in significant numbers by Jurkat-immunocasp-3 cells *in vitro*. The *in vivo* antitumor activity of immunocasp-3, which might be produced by tumors, muscle cells, and implanted Jurkat cells, was also demonstrated in a murine xenograft model of HER2-positive tumors. Unlike previously reported tumor killer proteins such as immunotoxins (22, 29, 30), caspases are human endogenous proteins that kill tumor cells in an intrinsic physiological manner, resulting in relatively weak immunogenicity and minor systematic toxicity over repeated treatments.

These results indicate that the immunocasp-3 molecule, which combines the properties of a tumor-specific antibody with the extreme proapoptotic potency of a caspase, can be secreted both *in vitro* and *in vivo* by modified tumor cells and normal somatic cells, including muscle cells and even hematopoietic cells. These modified cells remain viable if they are HER2 negative because immunocasp-3 is directed to the lumen of the endoplasmic reticulum and secreted cotranslationally (29, 30). Either direct injection of immunocasp-3 genes into specific tissues or infusion of gene-modified cells could be expected to suppress primary tumors and micrometastases because of continuous secretion of the killer protein and its diffusion through blood and lymph fluid. However, as indicated here, use of immunocasp-3 gene-modified autologous cells would likely provide a more convenient and effective approach to the treatment of HER2-overexpressing tumors, compared with direct administration of the chimeric effector protein, which involves a complicated procedure of protein purification. Indeed, such cell-based therapy, combining the specificity of antibodies, the potent cytotoxicity of apoptotic executioners, and the effector cell properties of lymphocytes such as homing and tissue penetration, possesses the advantages of both antibody-directed and cell-mediated immunotherapy. Results of the present study provide a platform for the development of novel therapeutic protocols for tumors that overexpress erbB2/HER2.

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